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SULFATED DEXTRAN—BLOOD ANTICOAGULANT ACTIVITY AND TOXICITY STUDIES IN ANIMALS*

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Dextran, the relatively new therapeutic agent, which according to several investigators[5, 7, 8, 12, 15] is effective in the emergency treatment of shock and in treating the nephrotic syndrome, may have further uses in the practice of medicine. The sulfuric acid esters of this compound are blood anticoagulants comparable to heparin.

Bergström[2] and other early investigators[3, 4, 9] have described the preparation and anticoagulant properties of sulfuric acid esters of several polysaccharide derivatives. Astrup *et al*[1] asserted that some of these compounds were unsuitable for clinical use because of the toxic effects noted in laboratory animals. Grönwall and co-workers[6] synthesized three sulfate esters of dextrans of varying molecular weights, and also found these preparations to be toxic in animals. Walton[14] demonstrated that sulfuric esters of low molecular weight dextran (20,000 or less) were free of toxicity, and he concluded that the toxic factor was related to molecular size.

A series of dextran sulfate esters was prepared by our laboratories. The anticoagulant activity of these preparations was studied *in vitro* and *in vivo*, and toxicity studies were undertaken in mice and rabbits.

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* Second ASMT Award Paper, presented at annual convention of ASMT, Louisville, Ky., June, 1953.

Experimental Preparation of Sodium Dextran Sulfate

The sulfation was achieved by adding powdered dextran to a mixture of pyridine and chlorosulfonic acid. The initial mixing was carried out at 20-25° C. and then the temperature was raised to 65-85°C, with continued vigorous agitation, for several hours to complete the reaction. The sulfated dextran was converted to its sodium salt and the product was purified and isolated by alcohol precipitation or by dialysis and lyophilization. This is essentially the same procedure used by other investigators.[6,11] Sulfur analyses were by a micro Carius procedure. Theoretically, sodium dextran monosulfate contains 12.1% S, and the disulfate 17.5% S.

IN VITRO Anticoagulant Activity

The *in vitro* anticoagulant activity of all sulfated dextran preparations was determined with citrated dog plasma. Physiologic saline was used as the diluent for the compounds to be tested, and the U.S.P. heparin assay procedure[10] was followed. No less than four concentrations of the test material were employed with each sample, and at the end of 1 hour anticoagulant activity was recorded in the following manner.

AC_0 = The highest concentration of test material allowing complete coagulation of 1.0 ml of dog plasma.

AC_{50} = The solution concentration at which approximately 50% coagulation takes place.

AC_{100} = The lowest concentration of test material completely inhibiting coagulation in 1.0 ml of dog plasma.

Sodium heparin (Lilly) was employed as a control for both the *in vitro* and *in vivo* studies. The results obtained in the *in vitro* experiments are shown in Table I.

The *in vitro* anticoagulant activity of the various lots of sulfated dextran appeared to be related to the degree of sulfation rather than molecular size. By referring to Table I, it is evident that all preparations within a range of 10.15% and 18.6% sulfur were active *in vitro*, while compounds of less than 10.15% sulfur (approximately one sulfate group per anhydroglucose unit) did not display significant anticoagulant activity; however, only two samples of low sulfur content were available for study.

IN VIVO Anticoagulant Activity

The blood anticoagulant effect of sulfated dextran was studied in New Zealand white rabbits. Following the administration of test material the animals were bled from the marginal ear vein, and the blood coagulation time was determined by the Lee-White method.[13] Results of the *in vivo* studies are summarized in the following tables.

TABLE I. SULFATED DEXTRAN—IN VITRO ANTICOAGULANT ACTIVITY

Sulfated Dextran Lot Number	Percent S	Approx. Mol. Weight	Micrograms/ml. Dog Plasma		
			AC_0	AC_{50}	AC_{100}
86-92	18.1	9,000	25	38	50
86-84	14.2	25,000	13	19	24
83-46	2.5	60,000	1000	....	....
84-28	11.7	60,000	50	63	75
84-32	17.1	60,000	40	60	80
84-35	17.8	60,000	20	25	30
85-52	15.2	60,000	30	40	60
85-57	14.9	60,000	25	38	50
85-59	13.0	60,000	12	32	50
85-68	12.0	60,000	25	35	50
85-96	10.15	60,000	37.5	50	75
86-85	18.6	60,000	15	19	23
87-07	13.6	465,000	25	38	50
SDS-6 (Native Sulfated Dextran)	8.9	2,000,000	3600	4200	4800
Sodium Haparin (Lilly)	....	17,000	2.5	3.5	4.0

Duration of the *in vivo* anticoagulant activity of sulfated dextran is apparently dependent on individual animal susceptibility as well as molecular size and degree of sulfation. Lot 86-92 (9,000 M.W.), by either the intravenous or intramuscular routes of injection in rabbits, displayed more prolonged anticoagulant activity than sodium heparin. Several sulfated dextran lots of average 60,000 M.W. produced very erratic results following intravenous injection. These were probably due to the irregular rate of excretion of high molecular weight material.

An oral dose of 357 mg/kg of sulfated dextran did not delay the coagulation time of a rabbit, and the oral studies were discontinued after administering to mice a daily dose of 5,000 mg/kg for 7 days without evidence of anticoagulant activity or gross pathology.

Intravenous administration of sulfated dextran in doses of 2-4 mg/kg did not produce any visible ill effects in rabbits. Massive doses by either the intramuscular or subcutaneous routes of administration produced internal hemorrhage, severe tissue damage and subcutaneous hemorrhage in the area of injection.

The Effect of Protamine Sulfate on the Anticoagulant Action of Sulfated Dextran

It is quite apparent that if a powerful anticoagulant is to be used effectively, there should be some means of blocking its action if the need arose. Chargaff *et al*[4] found that the anticoagulant effect of heparin and other substances both *in vitro* and *in vivo*, is destroyed by the protamine salamine. Therefore, the effect of protamine sulfate on the anticoagulant action of sulfated dextran was studied in rabbits. After the normal coagulation time was established by the Lee-White method,[3] dextran sulfate was

injected intravenously. The coagulation time was determined at 1 hour, and protamine sulfate was injected by the intravenous route immediately thereafter. Blood was again collected for coagulation tests following the injection of protamine sulfate.

TABLE II. SULFATED DEXTRAN—INTRAVENOUS INJECTIONS

Lot No.	% S	Approx. Mol. Weight	Dose Mg/Kg	No. of Rabbits	HOURS AFTER INJECTION				
					0	1	2	3	4
					Blood Coagulation Time in Min.				
86-92	18.1	9,000	2.0	2	12–15	75–84	39–51	28–30	15–29
86-84	14.2	25,000	2.0	2	9–10	45–51	–10–23	.....	.....
84-32	17.1	60,000	2.0	6	5–14	15–150	26–45	9–11	.....
84-35	17.8	60,000	4.0	3	5–11	46–60	20–35	6–50	6–22
85-52	15.2	60,000	2.0	7	6–12	12–90	12–66	5–21	.....
85-57	14.9	60,000	3.0	4	7–12	20–240	14–180	12–50	15–18
85-59	13.0	60,000	3.0	3	6–9	60–87	9–15	.....	.....
85-68	12.0	60,000	4.0	3	6–10	57–180	15–70	14–14	.....
85-96	10.15	60,000	4.0	3	5–9	18–70	17–51	14–21	.....
86-85	18.6	60,000	2.0	2	6–10	34–80	35–47	9–23	.....
87-07	13.6	465,000	3.0	3	6–12	66–300	15–36	7–13	.....
Sodium Heparin (Lilly)	.....	17,000	2.0	3	5–9	92–108	18–20	10–12	.....

The data in Table V indicate that protamine sulfate in a dose corresponding approximately to the weight of the injected sulfated dextran will destroy the anticoagulant action of sulfated dextran within 5 minutes. These studies seem to imply that the reversal action of protamine sulfate is an "all or none" phenomenon rather than a quantitative action, and future experiments on the mechanism are being planned by our labortary.

Acute Toxicity Studies

Intravenous toxicity studies were conducted in Carworth (CFW#1) mice and New Zealand white rabbits. The animals were observed for 7 days, and all were examined for gross pathology.

Sulfated dextran lot 86-92 (9,000 M.W.) was significantly less toxic in mice than the other compounds of higher molecular weight, but even the most toxic preparations provided a wide margin of safety. The intravenous toxicity curves obtained with compounds of average 60,000 molecular weight were very irregular, and it appears that molecular size is a toxicity factor. All deaths in these experiments resulted from severe internal hemorrhage; however, no gross pathologic lesions were observed in the survivors.

Subacute Toxicity Studies

Subacute toxicity studies were conducted with sulfated clinical dextran lot 85-96 (60,000 M.W.) in male New Zealand white rabbits. The test material was administered to one group of animals

TABLE III. INTRAMUSCULAR INJECTIONS IN RABBITS

Lot. No.	% S	Approx. Mol. Weight	Dose Mg/Kg	No. of Rabbits	HOURS AFTER INJECTION												
					0	1	2	3	4	5	7	24	31	48	55	72	79
					Blood Coagulation Time in Minutes												
86-92	18.1	9,000	12.0	2	6–14	33–325	260– > 380	> 320 > 320	.....	> 200 > 200	54– > 75	19–21	8–15	.....	.....	.....	.....
			120	2	6–6	> 1765 > 1765	1645 > 1825	1584 > 1765	.....	> 1700 > 1700	> 1500 > 1500	*. > 500	80	55	35	24	7
84-32	17.1	60,000	10	2	5–6	9	18–11	12–15	8–11	10–13	6–7	10–12	.....	.....	.....	.....	.....
			20	2	10–12	10–11	10–11	10–5	14–17	13–14	12–15	14	.....	.....	.....	.....	.....
			50	2	8–8	8–22	9–30	23–58	11–27	37–38	22–24	.....	.....	.....	.....	.....	.....
Sodium Heparin (Lilly)	...	17,000	10	2	6–10	10–10	10–45	10–22	14–90	13–57	12–21	14–17	.....	.....	.....	.....	.....

* One Rabbit Expired.

TABLE IV. SUBCUTANEOUS INJECTIONS IN RABBITS

Lot No.	% S	Approx. Mol. Weight	Dose Mg/Kg	No. of Rabbits	HOURS AFTER INJECTION									
					0	1	2	3	4	5	6	24	30	48
					Blood Coagulation Time in Minutes									
84-32	17.1	60,000	34	1	6	6	13	20	16	14	..	11	..	..
			40	1	6	..	..	15	..	..	50	113	6	..
			50	1	10	..	..	11	..	..	25	55	30	..
			60	1	9	9	18	27	40	47	48	180	..	9
			120	1	7	7	23	38	32	40	28	> 7 yrs.*	..	..
Sodium Heparin (Lilly)	...	17,000	10	1	9	32	50	50	55	48	24	15	..	..
			40	1	15	90	170	120	120	110	80	180	24	..

* This animal was sacrificed.

TABLE V. THE EFFECT OF PROTAMINE SULFATE ON SULFATED DEXTRAN ACTIVITY IN RABBITS

Sulfated Dextran Lot No.	% S	Approx. Mol. Weight	No. of Rabbits	SULFATED DEXTRAN-INTRAVENOUS			PROTAMINE SULFATE-INTRAVENOUS		
				Dose Mg/Kg	Pretreatment Blood Coagulation Time in Minutes	1 Hour Blood Coagulation Time in Minutes	Dose Mg/Kg	5 Min. Blood Coagulation Time in Minutes	1 Hour Blood Coagulation Time in Minutes
84-32	17.1	60,000	1	10	12	> 180	5.0	...	> 120
84-28	11.7	60,000	1	5	6	189	5.0	...	7
85-68	12.0	60,000	2	10	6–9	125–160	7.5	6–6	7–12
85-68	12.0	60,000	2	10	6–7	75–120	10	7–7	10–12

TABLE VI. INTRAVENOUS TOXICITY STUDIES IN MICE AND RABBITS

Sulfated Dextran Lot No.	% S	Approx. Mol. Weight	Test Animal	No. of Animals Per Dose	LD_0	LD_{50}	LD_{100}	IN VIVO Anticoagulant Dose Mg/Kg	IN VIVO Safety Index*
86-92	18.1	9,000	Mouse	10	500	1000 ± 150	2500	2.0	500
84-32	17.1	60,000	Mouse	5	50	82 (app.)	300	2.0	41
84-35	17.8	60,000	Mouse	5	20	57 (app.)	300	4.0	14.25
85-52	15.2	60,000	Mouse	5	100	215 ± 27	300	2.0	107.5
85-57	14.9	60,000	Mouse	5	50	140 ± 32	250	3.0	46.66
85-59	13.0	60,000	Mouse	5	150	194 ± 19	300	3.0	64.66
85-68	12.0	60,000	Mouse	5	50	205 ± 17	300	4.0	51.25
85-96	10.15	60,000	Mouse	5	50	220 (app.)	300	4.0	55
87-07	13.6	465,000	Mouse	5	50	80 (app.)	200	3.0	73.3
85-52	15.2	60,000	Rabbit	5	40	50 (app.)	200	2.0	25
Sodium Heparin (Lilly)	....	17,000	Mouse	5	150	220 ± 42	500	2.0	110

* Safety Index $= \frac{LD_{50}}{\text{In Vivo anticoagulant dose}}$

TABLE VII. SUBACUTE TOXICITY STUDIES IN RABBITS (SULFATED DEXTRAN LOT 85-96 (60,000 M.W.)

Rabbit No.	Route	Dose mg/kg	Total Daily mg/kg	Weight Kilograms		Pre-treatment	1st Day	3rd Day	7th Day	9th Day	10th Day	14th Day	Remarks
				Pre-treatment	14th Day		Coagulation Time in Minutes						
1	I.V.	6.25	19.75	2.5	2.37	13	8	6	5	4	9	7	.....
2	I.V.	6.25	19.75	2.25	2.08	6	8	5	5	5	7	5	.....
3	I.V.	6.25	19.75	2.55	2.34	7	7	15	5	15	10	10	.....
4	I.V.	12.5	37.5	1.8	1.50	7	10	5	5	5	5	7	.....
5	I.V.	12.5	37.5	2.5	....	7	25	30	..	..	..	..	Expired 5th day
6	I.V.	12.5	37.5	2.62	2.13	6	35	30	16	35	17	19	.....
7	I.V.	25	75	1.75	....	7	..	..	..	..	..	..	Expired within 24 hrs.
8	I.V.	25	75	2.0	....	7	..	..	..	..	..	..	Expired within 24 hrs.
9	I.V.	25	75	2.59	....	7	180	..	..	..	..	..	Expired within 24 hrs.
10	Subcutaneous	30	30	2.42	2.57	6	8	5	5	6	6	5	.....
11	Subcutaneous	30	30	2.9	2.8	11	5	8	3	3	5	8	.....
12	Subcutaneous	30	30	1.6	1.86	7	6	8	4	3	5	6	.....
13	Subcutaneous	40	40	2.38	....	8	5	..	..	..	..	..	Expired 3rd day
14	Subcutaneous	40	40	2.6	....	5	5	6	..	..	..	..	Expired 6th day
15	Subcutaneous	40	40	1.78	1.73	7	8	7	4	4	7	5	.....

intravenously, 3 times a day, for 5 days, treatment discontinued 2 days over the week end, then repeated for another 5 day period. A second group received daily subcutaneous injections for the same period. Weights were recorded daily, and blood coagulation times were determined 12 to 24 hours after the preceding injection throughout the experimental period. Normal values were established for erythrocyte sedimentation, hemoglobin, and erythrocyte counts; these tests were repeated at the end of the experiment. All animals were examined for gross pathology; and tissues were taken from the survivors for microscopic study.

During the subacute studies no evidence of cumulative anticoagulant activity was produced by repeated injections of sulfated dextran. Several animals did display increased blood coagulation times, however, this appeared to be related to the size of the dose rather than cumulative action of the test material. There was no significant variation in the erythrocyte sedimentation rate, but a decrease in hemoglobin and in the erythrocyte count was observed in all animals. The survivors of the intravenous group displayed weight losses ranging from 5 to 18%. These symptoms were probably due to excessive external bleeding following injections, or the collection of blood for the coagulation tests.

Gross examination of the survivors of subcutaneous injections revealed hemorrhage and subcutaneous tissue damage in the area of injection. Hematomas were noted at the injection sites of the survivors of the intravenous injections. Histologic examination of tissues obtained from the survivors of both groups did not reveal characteristic morphologic changes in any of the organs. All deaths occurring during the subacute test were due to severe internal hemorrhage following the repeated administration of large doses of sulfated dextran.

Summary

A number of sulfated dextran esters of varying molecular weights were examined for blood anticoagulant activity and toxicity in animals. As a result of these studies, it is evident that the *in vivo* anticoagulant activity was related to at least three factors:

1. Degree of sulfation of the compound; however, within a range of 1.2-2.5 sulfate groups per anhydroglucose units the activity is independent of the degree of sulfation.
2. Molecular size of the compound.
3. Individual animal susceptibility.

A wide margin of safety was observed between the effective intravenous anticoagulant dose of sulfated dextran and the LD_{50}. Repeated injections of these preparations in rabbits did not not produce cumulative anticoagulant activity, or characteristic morphologic changes in any of the organs. It was also noted that protamine sulfate in a dose approximately the weight of sulfated

dextran destroyed the anticoagulant action of sulfated dextran in rabbits within five minutes.

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BIBLIOGRAPHY

1. Astrup, T., Galsmar, L., and Volkert, M.: Polysaccharide sulfuric acid esters as anticoagulants. M. Acta Physiol. Scan. **8,** 215 (1944).
2. Bergström, S.: The active group of heparin. Naturwissenschaften **23,** 706 (1935).
3. Chargaff, E., Bancroft, F. W., and Stanley-Brown: The inhibition of blood clotting substances of high molecular weight. J. Biol. Chem. 115, 155 (1936).
4. Chargaff, E., and Olson, K. B.: The studies on the action of heparin and other anticoagulants. The influence of protamine on the anticoagulant effect *in vivo.* ibid **122,** 153 (1937).
5. Curry, C. A., and Mattox, D. M.: Personal communication.
6. Grönwall, A., Ingelman, B., and Mosimann, H.: A dextran sulfuric acid ester with heparin activity. Upsala Läkareforen. Förh. **50,** 397 (1945).
7. Ingelman, B.: Dextran and its use as a plasma substitute. Acta Scandinavia **1,** 731 (1947).
8. Ingelman, B.: Investigations on dextran and its application as a plasma substitute. Upsala Läkareforen. Förh. **54,** 107 (1949).
9. Karrer, P., Koenig, H., and Usteri, E.: Blood-coagulation-inhibiting polysaccharide polysulfuric esters and related compounds. Helv. Chim. Acta **26,** 1296 (1943).
10. Pharm. of the U. S. XIV, *272* (1950).
11. Ricketts, C. R.: Dextran sulfate—a synthetic analog of heparin. Biochem. J. **51,** 129 (1952).
12. Thorsen, G.: Dextran as a plasma substitute. Lancet **256,** 132 (1949).
13. Todd, J. C., and Sandford, A. H.: Lee and White's method for determining blood coagulation. Clin. Diag. by Lab. Meth. **10,** 199 (1943).
14. Walton, K.: Experiments with dextran sulfate as an anticoagulant. Proc. Royal Soc. Med. **44,** 563 (1951).
15. Wallenius, G.: The relief of nephrotic edema by dextran infusions. Scandinav. J. of Clin. Lab. Invest. **2,** 228 (1950).

LABORATORY METHODS FOR THE DEMONSTRATION OF L. E. CELLS*

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The manifestations of disseminated (systemic) Lupus Erythematosus are extremely protean and variable. Many patients are markedly toxic and skin lesions are classic symptoms. Formerly skin biopsy was relied upon heavily for laboratory confirmation of the disease; however, microscopic findings in acute cases are often equivocal and disappointing. The five classic changes recognized prominently in chronic discoid lupus erythematosus are seen in varying degree, if present at all, in acute cases. These cardinal changes in the skin are:

1. Hyperkeratosis with keratotic plugging.
2. Atrophy of the rete malpighii.
3. Liquefaction degeneration of the basal cells.
4. Perivascular infiltrate, chiefly lymphocytes, tending to be in the vicinity of dermal appendates.
5. Basophilic degeneration of collagen and elastic fibers.

Of interest to the hematologist is the fairly frequent finding of anemia, leukopenia, and thrombocytopenia. For these reasons, in 1946, Hargraves[2] of the Mayo Clinic began a study of the blood and bone marrow of patients with systemic lupus erythematosus.

Hargraves[2] invented no new technics or originated new methods, but simply applied well established procedures combined with careful observation and described a phenomenon which has had profound effect upon the science of medicine and has opened new avenues for the investigation of disease.

From the bone marrow of patients with systemic lupus erythematosus Hargraves[2] described a cell which he appropriately called the L. E. Cell. The L. E. Cell is a mature polymorphonuclear neutrophilic leukocyte which contains within its cytoplasm a large mass of material giving staining reactions suggesting its nuclear origin. This mass is usually round and so completely fills the cell that the nucleus is crowded to the periphery of the cell. It also appears that the material is derived extrinsic of the cell as this material is occasionally seen free upon the slide and often groups of polymorphonuclear cells are seen surrounding such masses apparenty phagocytizing the substance. This clumping of polys around this amorphous material is also part of the phenomenon and is appropriately called rosettes. It soon became known to Hargraves[3] that the L. E. Cell could not be found by many competent hematologists who were examining the bone marrows of patients with systemic lupus erythematosus and an

* Read before ASMT in Convention, Louisville, Ky., June 1953.

examination of his method revealed the cells were found only in bone marrow which had been mixed with an anticoagulant and kept outside the body approximately 30 minutes before making smears. The cells were most often found in the so-called concentrated smears from the buffy coat. This was one of the routine steps in examining all bone marrows aspirated.

Sundberg and Lick[4] reported finding the cell in the peripheral blood of three cases of diagnosed lupus erythematosus. In order to examine a large number of white cells, due to the leukopenia of the patients, they added an anticoagulant and concentrated peripheral blood and made smears from the buffy coat. Thus the basic steps for the demonstration of L. E. Cells were established. An anticoagulant was added to bone marrow or blood, this material incubated at room temperature outside the body for approximately 30 minutes, centrifuged, and smears made from the buffy coat. Bone marrow was preferable due to the large numbers of white cells it contained.

In 1949, almost simultaneously, Hargraves[3] and Haserick and Bortz[5] published their observation on the *in vitro* production of the L. E. phenomenon by mixing the plasma of patients with lupus erythematosus with the buffy layer from normal bone marrow. This demonstrated that the L. E. phenomenon was a property of the plasma and the L. E. cell merely an indicator of its presence.

Barnes, Moffatt and Weiss[6] showed that anticoagulants were unnecessary in the production of L. E. cells and they with Lane[7] devised an *in vitro* test for the L. E. phenomenon. Haserick[8] used bone marrow from experimental animals obtained from the physiology laboratory at his institution. Bone marrow mixed with proper amounts of heparin could be satisfactorily preserved in the refrigerator for eight to ten days and was a readily available source of polymorphonuclear leukocytes for performing *in vitro* tests for lupus erythematosus. Haserick et al [9,10] further showed that a factor was present in the gamma globulin fraction of blood plasma from patients with disseminated lupus erythematosus, which induced the formation of L. E. Cells and that this factor has the properties of an antigen. Gonyea, Kallsen, and Marlow[12] also Lee, Mitchell and Vural[14,15] demonstrated the formation of L. E. Cells in the clotting of the patient's blood. Here no additional cells were added, as the patient's blood furnished both the necessary constituents; that is, L. E. factor and polymorphonuclear leukocytes. Lee[14] further found that in all positive cases L. E. Cells were far more frequently encountered by the clot method than any other method.

With these basic facts numerous methods have been devised to produce and demonstrate L. E. Cells.[3,4,5,6,7,8,11,12,13,14,15,16,17] Many of these methods, exemplifying various principles, were

critically studied by Zimmer and Hargraves[18] who conclusively showed that L. E. Cell production is enhanced by a technic employing clotting of the blood. These authors summarize the L. E. phenomenon as being dependent on the presence of three substances.

1) The lytic or L. E. factor present in the serum gamma globulin of the patient with systemic lupus erythematosus.
2) A source of nuclear protein to react with the lytic factor. (In the clotted blood technics this source is the patient's own polymorphonuclear leukocytes.)
3) Viable phagocytic leukocytes to engulf the lysed material.

To facilitate the separation of cells from the fibrin clot, Magath and Winkle[17] devised an apparatus consisting of a fine mesh sieve of stainless steel wire gauze, 40 meshes to the inch. The blood clot is expressed through the sieve by means of a flat pestle. The liquid material is then centrifuged in a Wintrobe tube and smears made from the buffy coat and stained. Magath[17] also states that an ordinary dust or powder separation sieve of 40 mesh can be used.

We have employed the method of Magath and Winkle[17] on numerous occasions and found it to be completely satisfactory. With slight modification we have made the method adaptable to any laboratory wishing to carry out this test. To construct a sieve of Magath's and Winkle's[17] specifications would be quite impossible in many laboratories and of considerable expense if ordered from a machinist. In testing two sieves which were readily available with clotted blood from known lupus erythematosus patients it was found that 40 meshes to the inch was not entirely necessary as satisfactory L. E. Cell preparations were produced when cells were expressed from the clot by pressing the clot against a plate perforated with small holes and expressing the clot through a fairly large sieve mesh of 12 to the inch.

Equipment for the performance of this test has recently become available from a laboratory supply firm.*

A very satisfactory sieve for this purpose can be purchased for a few cents from the utensil counter of supermarkets, variety stores, and hardware stores. This sieve, as illustrated, commonly called a tea strainer, meets the specifications of Magath and Winkle[17] as it contains a flat stainless steel mesh of 40 meshes to the inch across the bottom. The sieve will fit into a 250 ml. beaker. The pestle is satisfactorily substituted by a small flat bottomed vial or test tube. The technic of the test is essentially that as outlined by Magath and Winkle.[17]

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* American Hospital Supply Corporation, 1210 Leon Place, Evanston, Ill.

This method will yield excellent smears frequently with little or no hemolysis in the Wintrobe tube. The cells are well preserved, often no distortion is evident. Hemolysis, when present, appears to have no effect on the appearance of the leukocytes.

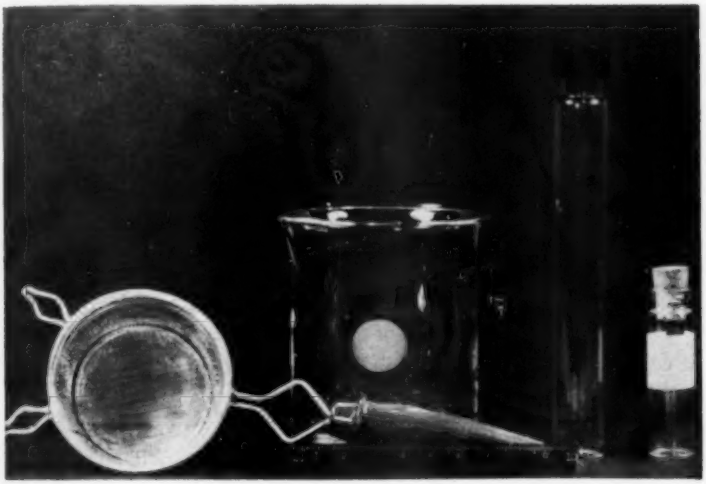

Fig. 1. Equipment for separating L. E. Cells from blood clot, including 40 mesh sieve, beaker, and flat bottomed glass vials.

Procedure

1) Draw 10 ml. of blood, place in a small cork stoppered, flat bottomed glass vial and allow to clot approximately two hours.
2) Rim clot if necessary and centrifuge 30 seconds at slow speed. Complete packing is not necessary.
3) Remove the serum and save, as it may be used to carry out other tests such as the Barnes-Moffatt[6] test.
4) Place the sieve in the beaker and turn clot into sieve.
5) Express cells from the fibrin clot with the flat base of the glass vial.
6) Fill a Wintrobe sedimentation tube with the blood, and centrifuge at 2000 rpm for five minutes.
7) Read the buffy coat in mm. and aspirate and discard all serum except for a volume equal to the buffy layer.
8) Aspirate the buffy coat and serum layer together, mix and make smears.
9) Stain with Wright's stain and examine.

The test has been utilized numerous times in our laboratories within the last year and five cases of acute disseminated lupus erythematosus have been studied.

We have not found the test to be positive in any case in which the bone marrow did not also yield L. E. Cells, although Hargraves[19] has warned against the possibility of false positive test with the blood clot methods. We use the criteria of Berman[20] *et al* in which at least 10 L. E. Cells per 500 white cells should be found to be called a positive test. Occasionally a rare cell interpreted as a Hargrave's "Tart cell" has been seen in preparations called negative. In one case on two occasions the bone marrow and the blood clot was positive for L. E. Cells, while the Haserick[5,9,11] test on the serum was negative.

Summary and Conclusions

1. Since the discovery of the L. E. Cell many technics have been devised to demonstrate this cell in blood and bone marrow. Of these tests the Magath-Winkle[17] technic is the most satisfactory laboratory procedure.

2. With the procedure here outlined this important test may be carried out with a minimum of equipment found in every laboratory plus the sieve, the expense of which is negligible.

3. False positive tests have not yet been encountered in our laboratories.

4. This test produced L. E. Cells whenever such cells have been demonstrated in the bone marrow and in one previously diagnosed case the Magath-Winkle test has given positive results when the serum tests with normal bone marrow were negative.

REFERENCES

1. Lever, Walter F.: Histopathology of the Skin. J. P. Lippincott Co., Philadelphia, 1949. Page 250.

2. Hargraves, M. M., Richmond, Helen, and Morton, R. J.: Presentation of Two Bone Marrow Elements: The "Tart" Cell and the L. E. Cell. Proc. Staff Meet. Mayo Clinic, 23:25-28 (Jan. 21) 1948.

3. Hargraves, M. M.: Production in Vitro of the L. E. Cell Phenomenon: Use of Normal Bone Marrow Elements and Blood Plasma from Patients with Acute Disseminated Lupus Erythematosus. Proc. Staff Meet. Mayo Clinic, 24:234-237 (April 27) 1949.

4. Sundberg, R. D., and Lick, N. B.: "L. E." Cells in the Blood in Acute Disseminated Lupus Erythematosus. J. Invest. Dermat. 12:83-84 (Feb.) 1949.

5. Haserick, J. R., and Bortz, D. W.: Normal Bone Marrow Inclusion Phenomena Induced by Lupus Erythematosus Plasma. J. Invest. Dermat. 13:47-49 (Aug.) 1949.

6. Barnes, S. S., Moffatt, T. W., and Weiss, R. S.: Demonstration of the L. E. Cell in the Absence of Anticoagulant. J. Invest. Dermat. 14:397-400 (June) 1950.

7. Barnes, S. S., Moffatt, T. W., Lane, C. W., and Weiss, R. S.: Studies on the L. E. Phenomenon. Arch. Dermat. and Syph. 62:771-785 (Dec.) 1950.

8. Haserick, J. R.: Plasma L. E. Test in Systemic Lupus Erythematosus. J. A. M. A. 146:16-20 (May 5) 1951.

9. Haserick, J. R., Lewis, L. A., and Bortz, D. W.: Blood Factor in Acute Disseminated Lupus Erythematosus.
   I. Determination of Gamma Globulin as Specific Plasma Fraction. Am. J. Med. Sc. 219:660-663 (June) 1950.

10. Haserick, J. R., and Lewis, Lena A.: Blood Factor in Acute Disseminated Lupus Erythematosus.
   II. Induction of Specific Antibodies Against L. E. Factor. Blood 5:718-722, 1950.

11. Haserick, J. R., and Bortz, D. W.: New Diagnostic Test for Acute Disseminated Lupus Erythematosus. Cleveland Clinic Quarterly 16: 158-161, 1949.

12. Gonyea, L. M., Kallsen, R. A., and Marlow, A. A.: The Occurrence of the "L. E." Cell in Clotted Blood. J. Invest. Dermat. 15:11-12 (July) 1950.

13. Moyer, J. R., and Fisher, G. S.: Experimental Production of "L. E." Cells. Am. J. Clin. Path. 20:1011-1015 (Nov.) 1950.

14. Lee, S. L., Mitchell, S. R., and Vural, I. L.: The "L. E." (Lupus Erythematosus) Cell. Am. J. Med. 10:446-451 (April) 1951.

15. Lee, S. L., Mitchell, S. R., and Vural, I. L.: The Lupus Erythematosus ("L. E.") Cells. Bull. New York Acad. Med. 26:266 (April) 1950.

16. Lee, S. L.: A Simple Test for L. E. Cells: Am. J. Clin. Path. 21:492-496 (May) 1951.

17. Magath, T. B., and Winkle, V.: Technic for Demonstrating "L. E." (Lupus Erythematosus) Cells in Blood. Am. J. Clin. Path. 22:586-587 (June) 1952.

18. Zimmer, F. E., Hargraves, M. M.: The Effect of Blood Coagulation on L. E. Cell Formation. Proc. Staff Meet., Mayo Clinic, 27:424-430 (Oct. 22) 1952.

19. Hargraves, M. M.: The L. E. Cell Phenomenon. Proc. Staff Meet., Mayo Clinic, 27:419-423 (Oct. 22) 1952.

20. Berman, L., Axelrod, A. R., Goodman, H. L., and McClaughry, R. I.: So-called "Lupus erythematosus inclusion phenomenon" of Bone Marrow and Blood. Am. J. Clin. Path., 20:403-418, 1950.

THE WORK OF THE REGISTRY OF MEDICAL TECHNOLOGISTS*

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To those of you who are the younger members of the American Society of Medical Technologists the year 1928 may seem to have been another era, the end of the "Roaring Twenties," the year before the stock market crash, or maybe the year your Mother and Dad were married. What happened then is pretty much ancient history to you; a quarter of a century ago! You may even visualize the medical technologists of those by-gone days as being tottery decrepit old men and women, retired from active duty because they couldn't hang onto a test tube, didn't have enough breath to use a pipette, and couldn't see well enough to do a blood count.

But to a good many of your very much alive, very active and vigorous members, 1928 was a year to remember, for it was in 1928 that the profession of medical technology was created and given status by the American Society of Clinical Pathologists. No longer was medical laboratory work relegated to the level of a maid's work. After having studied the question for two years, in 1928 the ASCP decided that there was enough demand for certification of medical laboratory workers to establish a certifying board, and so the Registry of Technicians was formed in that year as a standing committee of the Society. Thus, for the first time, medical technologists were recognized as a part of the medical team.

Recent evidence of the fact that medical technologists are considered a part of the team is the excellent cooperation offered to the medical technologists by the American Hospital Association in preparation for National Hospital Week. It is satisfying to know that among those recognizing medical technologists as members of the first team are such organizations as the AHA, the Catholic Hospital Association, the American College of Surgeons, the American Medical Association, the College of American Pathologists, and of course our own parent group, the American Society of Clinical Pathologists. The members of the ASCP have been concerned with your progress for a long time, and their concern has prompted them to set high standards for you to reach in becoming registered. They have given you a great deal of responsibility in carrying out the technical phases of medical laboratory work, and in the teaching of student medical technologists. You have been called various and diverse names—that lab girl, nurse, technician, technologist, technical assistant, the doctor's other hands, the doctor's extra hands.

* Read before ASMT, 1953, Louisville, Ky.

What you have been called does not matter. What does matter is that for twenty-five years there have been dedicated men and women who have gone quietly about their work, but in addition have done everything they could to raise the standards of laboratory work. Working side by side with the pathologists, you have accomplished much; you have envisioned much for the future of your profession. In many ways the first twenty-five years may have been the hardest, but that is not to say that the next twenty-five will be without their problems. Each year brings new problems. But with each year there are more of you to help solve these problems. And each year has shown steady progress.

Your various committees have reported their progress for the past year, and now, on behalf of the Board of Registry, it is my pleasure to report what has transpired in the Registry office.

Total Registration

The total number of medical technologists registered since 1928, including those registered as a result of the Spring examination of 1953 is 23,651, of which 18,586 are currently registered. During the year 521 registrants were withdrawn; 436 for delinquent fees and lost address, 30 deceased, 37 resigned, and 18 were withdrawn for violation of the Code of Ethics. There were 52 reinstatements, and it has been interesting to note that some of these were technologists who had been out of the field for several years, but for some reason or another wanted to go back to the practice of medical technology again. This bears out the Board's belief that medical technologists are not completely lost to the profession because they marry and have families, but that many of them do go back to it at a later date.

In the course of making a salary study this year, we also studied the records of medical technologists not working in any field, according to their renewal blanks. Of the 10,271 renewals studied, we found that 2,354 men and women medical technologists, or 22.9% of those studied, were not working in medical technology. Of these, 1,878 or 18% of the total number studied, were housewives. We went further and found that of the 1,878 housewives, 1,141 or 81% of the housewives had been inactive in the field from one to five years; 529 or 28% had been inactive in the field from five to ten years; 147 or 7.8% had been inactive from ten to fifteen years; 28 or 1.5% had been inactive fifteen or more years; and 33 or 1.8% had been inactive less than a year but more than three months. These women are still interested in their profession, and they continue to pay their renewal fees year after year. Some of them eventually will stop renewing their certification and will be withdrawn. But here are almost 2,000 women, not active in their chosen work, but still enough interested in that work to keep up their professional ties. They are

scattered all over the country, but wherever they are, it is likely that they have at least as much spare time as any of you have. Have you put them to work? Are they out recruiting for new students? Are they speaking at their clubs and their husbands' clubs about medical technology as a vocation? Are they handling publicity for your groups, addressing notices for meetings, doing a lot of the little odd jobs that will relieve you and make your local or state group more active, better organized? Or are they a potential you had not realized was there waiting to be tapped?

We noticed in our survey of the renewals that 88 men and women were working part-time in medical technology. There were only 4 men and they worked in hospitals, most of them while attending school, while 39 of the 84 women were working in hospitals and 28 in doctors' offices. The others were scattered in various other types of jobs.

There were 304 men and women who continued to renew their certification although employed full-time in other work. As in the case of the housewives, the greater number fell in the group which had been out of medical technology from one to five years; 44.7% of the women and 44.9% of the men. We also found that there was a small number of men and women who were not employed in any field, and the women were not housewives, but they continued to renew their certification. Most of these were students, some were ill, some caring for members of their family, etc.

Not counting those certified as a result of the last examination, we have certified 180 Histologic Technicians, 27 Bateriologists, 21 Chemists, 4 Specialists, and 86 Laboratory Aides. As you will see, there is more interest in the Histologic Technic classification than any of the others except medical technology. No Laboratory Aides have been examined for two years, and this classification will be included in the study being carried on by a committee appointed by the President of the American Society of Clinical Pathologists.

Many words have been spoken and written, many ideas set forth, many opinions expressed, many reasons given for the situation that now exists as regards the need for more medical laboratory workers. Some say the need can be met if we train high school students. Some say we must raise the qualifications to degree level, so that young people will feel that they are going into a profession; these people hold the opinion that a vocation is not a profession unless there is a degree attached. Some say that we must have aides of some sort, if only to do one or two phases of the work, and that on an assistant's level.

Examinations

In the past two examinations, 2,208 medical technologists were

examined for certification; of these, 1,797 passed and 411 failed. This is the first time since 1944 that there has been an appreciable drop in the number taking the examination, although in 1952 there were a few less than in 1951. The Spring 1953 examination was an experimental examination prescribed by the Board in an effort to find out some things we had not known before, and which could only be discovered by this means. The percentage passing the examination was higher than usual.

Schools of Medical Technology

The most recent list published by the Council on Medical Education and Hospitals of the American Medical Association shows that there are currently 538 Approved Schools of Medical Technology, an increase of 42 over last year's total of 496. During the year a few schools were discontinued, and some reported that they had no students. The 538 schools have a total student capacity of 4,116, an increase in student capacity of 528 over last year. Several new schools were approved at the June meeting of the Council; these are not included in this report.

I have mentioned the number of students taking the Registry examination and the number of schools and their student capacity in consecutive paragraphs because I want you to see the relationship of the two. Here on the one hand are 538 Schools of Medical Technology available to train a total of 4,116 students each year. On the other hand, during the past year we have examined a total of 2,208 candidates, not all of whom were recent students, and not all of whom were taking the examination for the first time. So it would appear that during the year the Board of Registry has examined not many more than half of the number of students it is possible to train in the Approved Schools. The Council reports that the actual number of students completing the course in 1952 was 2,063 for 435 schools, as compared to 2,220 in 1951. Everything points to the fact that, as I mentioned in my report to you last year in Portland, we are now feeling the effects of the low birth rate of the depression years; this is true in all fields, both professional and non-professional, laborers and all, alike. Young men and women are being wooed by business and industry in a variety of ways. Particularly men and women in the technological fields are being enticed to jobs with large salaries, with free expenses to visit prospective places of employment, and with all moving expenses paid to those who accept the jobs. Medical technology will have difficulty competing with offers such as these, because the greater majority of the medical technologists work in hospitals, where the salaries cannot be as high as they are in industry.

Is there any angle, then, that we can take in trying to interest young men and women of this year's high school graduating

classes in making medical technology their profession? We can point to the fact that salaries are increasing, apparently in about the same ratio that the salaries for teachers and nurses have increased. Aside from that there are the intangibles, the satisfaction gained from this kind of work, the importance of the medical technologist to the medical team, the fact that the future of medical technology seems promising.

During the past year thousands of pieces of vocational literature have been mailed from the Registry office. It is obvious that there is much rapidly increasing interest in recruitment of students so that the future of this profession will be assured. Quantity mailings, not including material sent with Registry exhibit, were:

Registry booklets	17,381
High School booklets	28,116
Approved School lists	10,761
SEVENTEEN reprints	19,685
OCCUPATIONAL TRENDS reprints	13,420
IF YOU WERE A MEDICAL TECHNOLOGIST	29,152
Total	118,515

This does not include 27,732 pieces of vocational material included with the Registry exhibit. This material was sent to State Societies of Medical Technologists, the Executive Office of ASMT, local societies, Approved Schools, pathologists, guidance teachers, colleges and universities. Selected pamphlets were sent to 2,710 people who wanted general information about medical technology. Marked booklets were sent to 1,728 who asked specific questions which could be answered by referring to certain passages in the booklet. In addition, an estimated 5,000 other letters included informational material.

Registry Exhibit

The Registry exhibit was used almost twice as much during the past year as it has been used in previous years, having been used at 22 meetings. These were medical meetings, meetings of medical technologists, career conferences, state fairs, hospital meetings, and several special meetings not falling into any of these categories. The Board is constantly striving to make the exhibit more interesting, without losing sight of the fact that it is an educational exhibit and must not only attract attention, but must tell the story of the work of the Board simply, in a dignified, professional manner. In spite of all the material that has been distributed, there are many who do not know what medical technology is, or even that there is such a profession. More important even than that, is the fact that there still are many

young people, as well as professional people, who do not know what is required to produce a good medical technologist. And so the exhibits we plan must perform two major jobs: 1) they must inform the public that there is a profession of medical technology; and 2) they must make sure that the public knows what constitutes proper training in medical technology, and what college background must precede that training.

After it has done that, the Registry exhibit goes a little beyond the actual limits of education regarding the field and its requirements, and it attempts to show the lay and medical public what medical technologists are earning, where they work, where they can become trained. It is hoped that your Society and the Registry can go ahead with our joint plans to make some sort of an educational exhibit available to more people during the course of a year, so that every person who wants to know about medical technology will have the opportunity to find out. This will take careful planning, but it is believed that it will be well worthwhile.

Motion Picture for Recruitment Purposes

For quite some time the Board of Registry and the American Society of Clinical Pathologists have been attempting to find the means to make possible a motion picture to be used in recruiting medical technologists. A tentative script was prepared about two years ago, and a committee appointed by the President of the American Society of Clinical Pathologists has been working on the project. I am happy to report to you that a major grant has been made by the American Cancer Society for this purpose, and we definitely expect that plans will go forward immediately toward the actual making of the picture. It will be a full-length picture, and should aid the recruitment program immeasurably. With this promised motion picture in mind, all of us must find outlets for distribution, and now is the time to plan these methods of distribution.

A Curriculum for Schools of Medical Technology

It is a pleasure to report that the third edition of Doctor Israel Davidsohn's CURRICULUM FOR SCHOOLS OF MEDICAL TECHNOLOGY is now available. This guide to the teaching and study of medical technology is much enlarged and expanded over the second edition, and the Board of Registry believes that the Approved School Directors and their technical assistants will find it an even better aid to their teaching than the previous editions. The Board is grateful to Doctor Davidsohn and his associates for their tireless efforts to make the CURRICULUM a worthwhile addition to the teaching programs in the Approved Schools. It is particularly appropriate that we make this announcement at your annual meeting, because Doctor Davidsohn

has asked the Board of Registry to turn over to the American Society of Medical Technologists his share of the Registry's payment to the authors for services rendered in preparing the new edition. When you see the CURRICULUM you will realize fully what a generous gesture Doctor Davidsohn has made to you in this gift, for it has been an enormous task to rewrite and expand and correct the manuscript, which is about twice as large as the second edition. Doctor Davidsohn has had to work on the CURRICULUM in his "spare" time, of which he has very little, since he is the immediate past president of the American Society of Clinical Pathologists, Professor of Pathology and Chairman of Department, Chicago Medical School; Director of Laboratories and Pathologist, Mount Sinai Hospital, and Director of Research, Mount Sinai Medical Research Foundation, Chicago, Illinois. The profession of medical technology and the American Society of Medical Technologists has no better friend than Dr. Davidsohn.

I have spoken at various times of the part that medical technologists play in the medical team that serves the patient. Probably some of you have read the report prepared by the Council on Medical Education and Hospitals of the American Medical Association as published in the May 9, 1953, issue of the Journal of the American Medical Association. In that report we find specific figures on the number of technical personnel in registered hospitals; this includes medical technologists, X-Ray technicians, physical therapists, occupational therapists, medical record librarians and other medical record personnel—a total of 55,217 full-time technical personnel. Of these, there are 21,373 full-time medical technologists, or 38.71 per cent of the total. There are 23,822 technical laboratory workers, including 2,449 on part-time assignments. Of this number, viz. 23,822, 8,612 were classified as medical technologists registered by the American Society of Clinical Pathologists. The next largest group is the group of X-Ray technicians, having a total of 12,412, a little more than half the number of medical laboratory personnel. It would appear, then, that we have the biggest job of all the technical personnel employed in hospitals, in supplying the need for workers. And so we come back to that all-important phase of our work, recruitment. As I said to you last year, the burden of recruitment rests with the American Society of Medical Technologists and the American Society of Clinical Pathologists. We must rise to the challenge so that the need for qualified medical laboratory workers can be met, and so that there will be no danger that the need will, instead, be met with poorly trained, substandard workers.

In the second edition of the booklet of information of the Registry of Technicians, published twenty years ago, we find this statement: "It is the endeavor of the Society (ASCP) to invest this useful calling with the dignity that it deserves, and to create a proper spirit of appreciation for the members of a vocation which is dedicated to the aid of suffering humanity." I believe that in the first twenty-five years of its existence the Registry of Medical Technologists has made great progress toward this goal. It is the hope of the Board of Registry, and I know it is your hope as members of the American Society of Medical Technologists, that in the next twenty-five years we can extend this goal so that the major portion of the medical laboratory workers will be included in our list of properly qualified medical technologists.

THE CULTIVATION OF VIRUSES: A REVIEW*

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PART II

Cultivation of Viruses in the Embryonated Egg

As an experimental animal for virus cultivation, the embryonated avian egg perhaps comes most closely to the ideal. The extra embryonic membranes, tissues, organs, and yolk sac combined provide a suitable environment for growth of a large number of viruses, all in a compact package which is normally sterile. Use of the developing egg in research with some viruses either reduces or eliminates the need for animals with the resulting savings in time, labor, and expense. The danger of cross infection is at a minimum. The egg has been shown to be incapable of reacting to foreign protein growing on the chorioallantoic (CA) membrane,[38] and has also been shown incapable of complement production until either just before or just after hatching.

As in every experimental animal, there are limitations. Nonspecific irritative lesions sometimes parallel closely those of virus origin. In some cases, examination of the embryo will not suffice to determine whether growth of the inoculated virus has taken place. Bacteriologically sterile inocula must be used, a limitation which can be partially overcome with the use of penicillin and streptomycin to eliminate bacterial contamination.[39] Another limitation, and perhaps the most important of all, is that to date there are viruses which will not grow in the embryonated egg. Perhaps, as time passes and modifications of the existing techniques or new techniques are developed, this limitation will be largely overcome.

History of Virus Cultivation in the Embryonated Egg

In 1906 Levaditi[66] reported the work of Borrel and supplemented his work by further investigation of an acute spirillum septicaemia which developed in the albumen of an embryonated egg as a result of inoculation with the blood of an infected fowl. This investigator also noted that eggs from fowl immune to the disease, were also immune to this disease.

Rous and Murphy,[40] working with a highly malignant strain of avian neoplasm, first found that the active agent was present in cell-free filtrates and further proceeded to cultivate it in poultry and then in developing embryonated eggs. Murphy[41] later cultured the Jensen rat sarcoma on the CA membrane of a developing embryonated egg (hereafter referred to as egg). Perhaps it was unfortunate, but the work on eggs following

* Part III to follow in November-December, 1953, American Journal of Medical Technology. (See July-August issue for Part I.)

that of these two investigators centered around that of tumor grafting rather than the pursuit of problems of general infection.

Juan and Staub[66] injected the virus of avian pest into an egg and demonstrated six passages before death of the virus. In 1929 Gay and Thompson,[42] working with vaccinia, injected the yolk sac of eggs with two positive passages resulting. These experiments were based on the assumption that the viruses were able to multiply in a cell-free medium.

Goodpasture and his collaborators were the first to emphasize the potentialities of the avian egg for the investigation of the infectious properties of virus. Woodruff and Goodpasture,[43] in 1931, found that the ectodermal and endodermal cells of the CA membrane of the chick egg were susceptible to infection with the virus of fowl pox. Soon after, Goodpasture, Woodruff, and Buddingh[44] infected the CA membrane with a strain of Levaditi's neurovaccinia and with the virus of *Herpes simplex*. Also they noticed that the chick embryo was susceptible to viruses which were only slightly pathogenic to adult fowl. In 1934, Goodpasture and Buddingh[45] used smallpox vaccines successfully which were prepared on the CA membrane of embryonated eggs.

In 1933, Burnet started a series of publications which expanded the scope of egg inoculation for virus work. Most notable of his contributions was the technique for dropping the CA membrane by creating an artificial air space. This technique enlarges the area of the CA membrane available for inoculation, and has made possible the pock-counting technique for virus titration.

Allantoic and amniotic inoculations were initiated by Goodpasture[46] and further developed by Burnet,[47] and Nigg, Crowley, and Wilson.[48]

Nigg et al,[49] following work done previously by Cox with the rickettsiae, utilized the yolk sac of the egg for the production of influenza virus for complement fixation tests.

The intracerebral route was first used by Buddingh and Polk[50] in 1939. Two years later Dawson[51] adapted the virus of rabies to grow in the cerebrum of the chick embryo.

Eichorn,[52] in 1940, described the technique for intravenous inoculation of chick embryos. His work was a development of the original work by Polk, Buddingh, and Goodpasture.[53] No extensive application for the intravenous route of inoculation has been found.

Materials and Equipment

Some special equipment is necessary for chick embryo cultivation. The amount and type will vary with the needs of the individual laboratories.

An incubator, preferably one suitable for commercial hatching, is necessary for egg incubation. All electric incubators have proven most satisfactory. They should be equipped with automatic humidity controls, an automatic egg turning device, and the usual automatic heat regulating thermostat. The temperature of the eggs before inoculation should be kept in the range of 37 to 38° C., with the relative humidity regulated between 40 to 65 per cent.

If inoculation does not disturb the embryo, the eggs can be returned to the commercial incubator; otherwise they are placed in a bacteriological incubator in a fixed position and are not turned during subsequent incubation.

A dependable supply of eggs is essential. It is felt by some workers that white shelled eggs are preferable to darker shells because more of the embryo can be seen by candling. White eggs are especially advantageous with intracerebral and intravenous inoculations. High fertility of the eggs is desirable. Clean eggs are essential. Freedom from infection by *Salmonella pullorum* can be insured by procuring eggs from tested pullorum-free flocks.

Since the role of natural immunity in the embryonated egg is not known,[54] it perhaps would be well to secure eggs from flocks known to be not immune to the virus being cultivated. However, work has been and is being done especially with the virus of Newcastle's disease, cultivating viruses on eggs from immune fowl.

Beveridge and Burnet[54] have designed racks on the shape of isosceles triangles, with sides four inches long and a base of two inches, for holding eggs during manipulation. Eli Lilly and Co.[55] use commercial egg slicers with the slicing device removed. Any type of rack which will hold an egg in a fixed position is workable. Racks used for inoculation of eggs should be constructed of material which will withstand repeated autoclaving.

A suitable candler can be made from a light and a reflector, or can be purchased commercially.

Different types of drills are used for cutting the shell. Perhaps the one most commonly used is a dental drill equipped with a carborundum abrasive disk. These drills are rheostated and speed is controlled by a foot treadle. Blundell,[56] in 1944, suggested the use of a regular rotary tool equipped with a suitable carborundum disk. The tool is mounted in a position so that the operator can hold an egg in his hands, with his hands resting on the table, and apply it to the tool. Blundell found that, because of the rest, it was possible to drill the eggs with greater accuracy.

Several small pieces of equipment are necessary. There must be available material such as paraffin, wax, scotch tape, cellophane, cover glasses, or some other suitable device for closing

eggs after inoculation. Dissecting needles are necessary for breaking the shell membrane. Inoculation of eggs involves the usual array of syringes, needles, tubes, and racks.

Management of Eggs Before Inoculation

Only clean eggs should be used. Since washing destroys the gelatinous shell coating of the eggs, it makes them more susceptible to contamination. The eggs are incubated at 37 to 38° C. for the desired number of days prior to inoculation, and then are candled. Candling is necessary to insure the inoculation of only the eggs which contain live embryos. The embryo appears as a dark shadow in the egg. Movement can be seen, and in the older embryos it is the best indication of life. Infertile eggs and those with dead embryos are discarded. At this point the egg is marked according to the inoculation procedure to be used. The egg is then drilled. The marking of the egg and the type of drilling necessary will be discussed under the various routes of inoculation.

Prepartion of Inoculum

Since spore-forming saprophytes and pathogenic bacteria grow readily in the egg, great care must be taken to see that the inoculum is bacteriologically sterile. Antibiotics such as penicillin and streptomycin are used to control these bacterial contaminants. Occasionally the inoculum must be filtered through bacterial filters to insure bacterial sterility.

When an embryo is harvested a portion should be inoculated into a suitable medium to check for bacterial sterility.

Inoculation by Various Routes

Aseptic technique must be maintained throughout the inoculation procedure. Some laboratories use a semi-sterile room for inoculation, but most workers have experienced little trouble from air-borne contamination in an open working space. This is probably due to the natural resistance of the egg. Opinion varies as to the value of shell sterilization by application of alcohol or iodine. Some laboratories use application of a suitable disinfectant before an egg is inoculated whereas others do not.

Preparation and Inoculation of the Chorioallantoic Membrane (CA)

The CA membrane is composed of three layers: ectoderm, mesoderm, and endoderm. Because of the availability of these three germ layers to an inoculated virus, the viral propagating powers of this membrane are large. The most desirable embryo age for CA inoculation is from 11 to 12 days, but younger embryos are sometimes used for the cultivation of special viruses. There are several methods of drilling to expose the CA membrane. Three will be discussed here.

Window Method. A window is cut in the side of an egg so that a square of the shell can be lifted out. This is done by penetrating the shell with the drill and enlarging the penetration point by grinding against the uncut edge. When three sides of the square have been cut, the flap of shell is forced upward with forceps and torn off. If a large area of CA membrane is needed for inoculation, the membrane can be dropped. This is done by drilling a hole of small diameter over the air sac. In all drilling operations, care must be taken to prevent the cutting of the shell membrane. Once the hole is drilled over the air sac, the shell membrane is punctured and a syringe, bulb, or some other source of a gentle vacuum is applied over the hole. The vacuum evacuates the air space and the weight of the embryo causes the contents of the egg to fill the air space. This in turn results in the CA membrane falling away from the shell membrane at the top portion of the egg.

Triangle Flap Method. The triangle flap method is essentially the same as the window method with the exception that a triangular opening is made instead of the rectangular window. The triangle is equilateral and is approximately 12 cm. on each side.

Air Space Method. The air space is noted by candling and marked. A drill is used to cut around the circumference of the egg approximately ¼ of an inch nearer the air space end of the egg than was the line denoting the position of the air space. The shell is pierced by the drill at one point to facilitate the entrance of forceps for breaking the shell. When the shell is removed, a wide expanse of shell membrane is exposed. Directly beneath this is the CA membrane. The CA membrane is not dropped in this method.

The inoculation technique is the same in all methods. The egg is placed in a suitable stand and a drop of sterile saline may be placed on the shell membrane to make it transparent. The shell membrane is pierced carefully to expose the CA membrane. Care must be taken that the CA membrane is not damaged in this operation. One suggested method of inoculation is to lay the point of a needle on the shell membrane at right angles to the fibers which run obliquely across the egg. The needle is then pressed downward and backward and a slit is made in the membrane along the line of the fibers. A drop of saline is placed over the slit so that it will run in between the shell and CA membranes. The membrane is then dropped.

Hemorrhage has a tendency to produce non-specific lesions. Therefore, care must be taken to prevent this from happening.

The slit in the shell membrane is lengthened and a portion can be torn out or laid over so as to be out of the way. Inoculation can be accomplished by a Pasteur pipette, a previously calibrated capillary pipette, or by the use of a suitable syringe.

Small pieces of tissue can be used for inoculation by rubbing them gently over the surface of the CA membrane.

There are many methods for sealing the holes made in the shell. The hole made for dropping the embryo is most easily sealed with a drop of melted paraffin. The window can be sealed with a cover slip and paraffin, scotch tape, cellophane and sterile albumen, or perhaps most simply with a good grade of filter paper sealed with paraffin.

After the eggs are sealed, they are placed in an incubator with the window up. With this type of inoculation they are not turned.

Another technique, a modification of that of Alexander[57] and Dunham,[58] has been used with considerable success. The feature of this modification is the reduction of the amount of manipulation necessary for the CA inoculation with a subsequent decrease in non-specific lesions. A small hole about 3 by 2 mm. is made by applying a drill to the egg shell at an angle. The immediate area around the hole is covered with paraffin, and sterile liquid paraffin is applied to the shell membrane as a clarifier. The inoculum is dropped onto the paraffin over the shell membrane and the membrane is punctured through the drop of inoculum. The CA membrane is then dropped as it is for the conventional type of inoculation. As the embryo drops, the vacuum produced draws the inoculum through the shell membrane and it is deposited on the CA membrane. A drop of melted paraffin is used to seal the hole made. Results have shown that the inoculum is well distributed over the surface of the CA membrane. The eggs are then ready for reincubation.

Low humidity is occasionally advantageous for CA inoculation in that the air space in the egg enlarges more rapidly under this condition and will subsequently present a larger area of the CA membrane available for inoculation when the embryo is dropped.

Examination of Lesions

If a coverslip is used to seal the opening made for CA membrane inoculation, the progress of lesion formation can be watched as the incubation period progresses. Goodpasture described a technique for watching lesion formation, using the Leitz "Ultropak" equipment. Himmelweit[59] refined the technique. The CA membrane was dropped and inoculated in the conventional manner, then was sealed with a coverslip except for a small opening in the paraffin. The natural air space was then restored by forcing air gently into it. This raised the CA membrane into contact with the coverslip. The small vent hole in the paraffin around the coverslip was then sealed. It was found that high-power microscopy of the growing tissue was possible with this technique.

Harvesting

Harvesting can be done with the least danger of contamination by drilling along the circumference of the long axis of the egg so that the top of the egg can be removed to expose the embryo. Paraffin wax is placed along the drill cut before the top portion of the shell is lifted off. The shell membrane is broken with a sterile dissecting needle, a small part being left to act as a hinge for the portion of shell to be lifted. The CA membrane, allantoic fluid, amniotic fluid, yolk sac, and embryo are exposed and so lend themselves to harvest.

Pock-Counting Technique

With viruses that form lesions on the CA membrane, it is possible to count the number of lesions produced and thereby compute the number of infective viral particles present in the inoculated dose. The CA membrane is inoculated in the usual manner and incubated for a period which varies with the virus being cultivated. The membrane is harvested and the lesions counted.

Although the method is simple, there are difficulties encountered by the interpretation of non-specific lesions and secondary foci, especially with the viruses which produce small lesions. Secondary lesions are caused by moisture washing virus particles from a primary lesion to another place on the membrane. The primary lesions are normally larger than the secondary. Experience in the method is necessary for accuracy.

Inoculation of Foreign Tissues by Graft on the CA Membrane

Many authors have described methods for grafting foreign tissue on the CA membrane of a developing egg. This procedure can best be used to increase the scope of the egg's ability to propagate viruses.

TenBroeck[60] used this method to grow the virus of hog cholera in minced swine testes placed on the CA membrane. He mixed blood from infected animals with minced swine testes and Tyrode's solution, and deposited them on the CA membrane with a calibrated pipette. The eggs were reincubated at 37° C. for three days, opened, and the CA membrane with the pieces of testes were harvested and ground. The virus was maintained for 13 passages.

Goodpasture and Anderson[61] grafted human skin, amnion, and chorion, on the CA membrane and successfully grew the viruses of herpes simplex, variola, and vaccinia.

Amniotic Inoculation

The eggs are candled in the usual manner and, as an aid to inoculation, the point of origin of the allantoic vein is marked on the shell. This point is found near the air space and is recog-

nized by a shadow of the allantoic vein at the junction of two large veins. From this point the site for amniotic inoculation is determined by holding the blunt (air sac) end of the egg toward the operator and projecting a straight line along the long axis of the egg to the point at which the egg reaches its greatest diameter. The site of inoculation is marked ½ inch in a clockwise direction from this point.

The embryo will lie directly under the point marked in all but a few eggs. The egg is drilled at the point marked in the same manner as it is for window inoculation of the CA membrane.

The age of the embryo varies for different viruses. Eggs from 7 to 15 days have been used. The embryo begins swallowing movements at approximately the 13th day. This increases its susceptibility to some viruses because this swallowing facilitates the passage of virus from the amnion to the lung and respiratory epithelium of the embryo. However, in some cases, the increase in susceptibility of the embryo because of the availability of the respiratory epithelium is more than offset by the cells of the embryo being less susceptible because of their age. Hence the wide range of ages used for this type of inoculation.

Inoculation is done by first dropping the CA membrane. Then a syringe with a 22 or 23 gauge needle is introduced through the CA membrane as near to the yolk sac as possible without piercing it. As the needle is introduced, pointed toward the embryo, the pressure will pull the yolk sac over slightly. As this is done, the weight of the yolk sac will facilitate the piercing of the amnion by the needle. Beveridge and Burnet[54] suggested that the CA membrane be cut and the amnion be grasped with forceps and then inoculated. Closure of the shell is exactly the same as in CA membrane inoculation.

Hirst[62] suggested a different technique for amniotic inoculation. Thirteen-day embryos were inoculated over an egg candler. The eggs were rotated until the shadow of the embryo appeared near the upper surface of the egg. A puncture was made over the air sac and one directly over the embryo. Inoculations were made by inserting a 22 gauge needle one inch long through the hole made over the embryo and by impaling the embryo with a quick thrust of the needle. As the embryo was impaled it would disappear and would reappear as the needle was withdrawn from the egg. The holes were sealed with paraffin.

Pathological changes of the embryo from inoculation of the amniotic fluids are mostly non-specific. Burnet described these changes, after three-day incubation with influenza, as retarded development, weak circulation, anemia, and death in some cases. Tracheal fluid appeared turbid to the eye and stained smears showed vacuolated epithelial cells, leucocytes, and immature nucleated red blood cells. The lungs were whiter and firmer

than normal.

Proof of viral multiplication can be shown by inoculation of susceptible animals; or, if the virus is influenza, vaccinia, ectromelia, or Newcastle's, by Hirst's haemagglutination reaction.

Collection is best facilitated by exposure of the embryo as described under CA inoculation. The CA membrane is clipped away and the allantoic fluid of the embryo discarded. The embryo is grasped with forceps and a sterile Pasteur pipette is thrust into the amniotic sac and the fluid collected. Tracheal fluid for smears can be collected from the embryo by using a fine capillary pipette.

Yolk Sac Inoculation

Embryonated eggs of 5 to 6 days incubation are used commonly in yolk sac inoculations. They are candled in the usual manner and a small hole is drilled in the air sac end of the egg leaving the shell membrane intact.

Inoculation is usually done with a syringe equipped with a 20 to 22 gauge needle. The needle is inserted through the drill hole, is thrust directly inward along the long axis of the egg for 2 to 3 cm., and the inoculation is made. The hole is covered with paraffin and the eggs are incubated at a suitable temperature for the infectious agent inoculated.

The yolk sac is harvested by first cutting the shell again as in CA harvesting or by removing the air sac end of the shell. The membranes are cut along with the umbilical stalk. The embryo is picked out with sterile forceps and discarded and the yolk sac is removed from the shell and placed in a sterile petri dish. Small pieces of the sac can be cut off for smears if desired and the remaining portion is transferred to a sterile tube. The infectious agent can be liberated by grinding.

With some viruses, e.g., psittacosis, the titer of the yolk is the same as that of the membrane. In this case, harvesting of the infectious material is most easily done by inserting a hypodermic needle into the exposed yolk sac and withdrawing the yolk.

After inoculation the eggs should be candled twice daily for evidence of death of the embryo. The yolk is harvested as soon as possible after death of the embryo. In most cases there will be little or no microscopic change of an infected embryo from the normal. Smears must be made and stained and examined for typical lesions if any are produced by the virus inoculated. Hirst's phenomenon can be used to demonstrate multiplication where applicable. If these methods are of no value, then the presence of the infectious agent must be shown by inoculation into suitable animals.

Allantoic Inoculation

Usually eggs which have been incubated 10 to 11 days are used for allantoic inoculation. These are candled and a point is selected where the CA membrane appears to be well developed and free from large blood vessels. Best development of this membrane is usually on the side of the egg opposite the line along which the edges of the growing membrane have fused. The eggs are drilled so that a small groove from 2 to 3 mm. long and 1 mm. wide is shaped. Care must be taken not to injure the shell membrane.

Two methods of inoculation are in common use. One utilizes a syringe and needle which is used to pierce the shell and CA membranes. The needle is then thrust in from 2 to 4 mm. and the inoculum discharged. The other method is perhaps more convenient. The area around and in the groove is swabbed with hot paraffin and the inoculum is placed over the area of paraffin covering the groove in the shell. Then a dissecting needle is used to puncture the paraffin, shell, and CA membrane. When the needle is removed the inoculum will flow through the hole. The inoculum usually flows into the egg rapidly and only occasionally will it be necessary to stab a new hole in the membrane to expedite the introduction of the inoculum.

The allantoic fluid is collected by removal of the shell over the air sac, tearing the membranes, and pipetting off the allantoic fluid. The eggs are usually incubated 36 to 48 hours prior to harvesting of the allantoic fluid. Refrigeration of the eggs just prior to harvest will minimize hemorrhage during harvest.

Francis and Salk[63] have developed a method for allantoic harvesting of influenza virus which utilizes the adsorption of influenza virus on red blood cells. They inoculated 0.1 ml. of 1/1000 dilution of infected allantoic fluid, diluted in physiological saline, into the allantoic fluid of 11 or 12 day old embryonated eggs. Harvest was 48 hours later. As in the conventional procedure, the shell was removed along with the underlying membranes. Then the blood vessels were torn and, while the embryo bled, the egg was rotated so as to discourage clotting. The bloody fluid was aspirated with a needle and collected in centrifuge bottles immersed in an ice water bath. The fluid was then centrifuged in a chilled centrifuge for three minutes at 500 to 1000 rpm and the supernatant fluid is poured off. The cells were washed with cold physiological saline, decanted, and then more saline was added. It is important to keep the temperature from 4 to 8° C. for the preceding steps because when the temperature rises virus elution occurs. The cells plus the desired amount of saline are then put into a 37° C. water bath and agitated gently until the temperature rises. Then the suspension is incubated at 37° C. for 2½ hours.

During the incubation the virus is eluted from the red blood cells so that, after a final centrifugation, the supernatant fluid is the source of the virus and the red blood cells are discarded. Francis and Salk found that they could demonstrate tenfold increases in virus titer consistently with this method.

There are no specific changes in the allantoic fluid which indicate growth of the infectious agent. Death of the embryo may occur according to the nature of the virus being cultivated. Demonstration of increase in viral titer may be shown by hemagglutination if applicable, or by inoculation into suitable animals.

Intravenous Method

Eight day old embryos are excellent to use but difficult to inoculate, whereas ten to eleven day old embryos can be inoculated with relative ease. Hence, the age of the embryo used is determined partially by the skill of the person inoculating. The eggs are candled and a vein is located and marked to show location and direction of flow. The shell membrane is exposed by using the window or shell flap method and a drop of sterile mineral oil is applied to the shell membrane to make it transparent. Eichorn[52] found that a 25 to 27 gauge needle in a ¼ ml. tuberculin syringe was most advisable for use in inoculation.

The needle is inserted, at an acute angle with the bevel held uppermost, in such a manner that the inoculation will be made in the direction of the flow of blood. The inoculation is made and the needle is withdrawn carefully to prevent hemorrhage. Since hemorrhage is more likely with veins larger than the diameter of the needle, it is better to inoculate veins slightly smaller than this diameter. The egg is then sealed with wax, paraffin, or some other suitable material. Eichorn used a six per cent solution of paralodion in ether.

Bleeding can be done in exactly the same manner as the inoculation, except that the needle is inserted against the flow of the blood. If the embryo is to be sacrificed, it is easier to remove it from the shell and obtain blood directly from the exposed heart.

Mortality in Eichorn's studies was 30 per cent. However, he suggested that this figure could be reduced with improved technique.

Intracerebral Inoculation

Usually 8 to 14 day embryos are used. Candling and drilling procedures are identical with those of amniotic inoculation. Needles for inoculation vary from ½ inch-27 gauge to 1½ inch-22 gauge. The smaller the needle used, the easier it is to penetrate the skull of the embryo. Usually the head of the embryo will be visible but, if not, it can be brought into view by manipu-

lation with the needle. Inoculation is done by a short sharp thrust into the skull and the discharge of the syringe contents. Brain inoculation can be more precise if the head is held in place by grasping an eyelid or the beak of the embryo. Mortality from other causes than the virus inoculated varies from 10 to 40 per cent, and usually takes place in the first 48 hours after injection. Embryos which die in this period are therefore discarded.

Dawson[51] found that the pathological changes associated with rabies in the chick embryo were not observable until the third or fourth passage. Then hydrocephalus became severe and the entire embryonic ventricular system was dilated. Negri bodies were present. Anderson[64] found that *Herpes simplex*, after 24 hours, caused localized changes such as meningitis at the site of injection. At the 72 hour period, extensive hydrocephalus with large areas of hemorrhage and necrosis were present in the cerebrum.

Harvesting is best done by removing the head of the embryo and exposing the brain by dissection. Sections can be taken for histological study and the remainder ground for recovery of the infectious agent. Growth of the inoculated virus is demonstrated by characteristic lesions and by inoculation into susceptible animals.

Other Methods of Inoculation

De-embryonated eggs. Bernkoph[65] has developed a technique using de-embryonated eggs which, in reality, is quite similar to tissue culture techniques. Eggs of 14 to 15 days incubation are candled and drilled as in the air sac method of CA inoculation. The shell and CA membrane are cut away and the embryo with its yolk sac is removed after cutting the connection between the embryo and the chorioallantois. All remaining tissues, except the CA membrane, are washed out with sterile cold isotonic saline, and the egg is filled with inoculated Tyrode's solution containing 10 units of penicillin and 40 micrograms of streptomycin per milliliter. A sterile rubber cap is used to seal the opening in the egg. The inoculated eggs are placed in a roller tube rack and incubated.

Intramuscular inoculation. The same technique is used for intramuscular inoculation as for intracerebral, including the methods for preparing the egg. The only exception is that the inoculating needle is thrust into the muscle of the embryo instead of into the skull.

Albumen inoculation. The egg is candled and a small hole is drilled in the end of the egg opposite the air sac. The inoculum is injected through the shell membrane directly into the albumen of the egg.

Many viruses have been cultivated in the chick embryo and,

as investigations proceed, undoubtedly more viruses will be found that are cultivatable. The following table lists some of the more common viruses which have been cultivated in embryonated eggs. Material for this table was adopted from Beveridge and Burnet,[54] and Rivers.[66]

Virus	Route of Inoculation and age of Embryo most Commonly Used (in days)
B. virus	CA 10–12
Colorado tick fever	Yolk sac 7–8
Common cold	CA 10–12
Cowpox	CA 10–12
Dengue	Amnion 5–6
Durand's disease	CA 10–12
Ectromelia, infectious	CA 10–14
Encephalitis, Japanese B.	CA 10–12
Encephalitis, St. Louis	CA 10–12
Encephalomyelitis, equine	Embryo 7–9
Encephalomyelitis, mouse	Intracerebral 11–13
Fowl plague	CA 10–14
Fowl pox	CA 10–14
Hepatitis, infectious	Amnion 7–8
Herpes simplex	CA 12–14
Herpes zoster	CA–human skin grafts
Hog cholera	CA–minced hog testes
Influenza	Amnion, allantois 10–13
Keratoconjunctivitis	CA, yolk sac
Laryngotracheitis	CA 10–14
Louping ill	CA 10–12
Lymphocytic choriomeningitis	CA 11–12
Lymphogranuloma inguinale	Yolk sac 6–8
Mare abortion	CA–human amnion graft
Measles	CA 10–12
Meningopneumonitis	CA 10–12
Mumps	Allantois 8–12
Myxomatosis, infectious	CA 10–14
Newcastle disease	CA 10–14
Pacheco's parrot disease	CA 10–14
Pneumonia, atypical	Amnion 10–12
Pneumonia, cat	Yolk sac 5
Pneumonitis, mouse	Yolk sac 5–7
Pseudorabies	CA 12–14
Psittacosis	CA 9–10
Rabies	Brain, yolk sac 7–9
Rift Valley fever	CA 10–12
Rous sarcoma	CA 10–14
Sheep bluetongue	Yolk sac of eggs from riboflavin-deficient hens
Sheep pox	CA 10–14
Smadel's pigeon virus	CA 10–14
Stomatitis, vesicular	CA 7–13
Vaccinia	CA 10–14
Variola	CA 10–12
Yellow fever	Embryo 6–8

REFERENCES

38. Murphy, J. B. Studies in tissue specificity II and III. J. Exp. Med., 19, 181, and 513.
39. Rose, H. M., Pearce, E., and Malloy, E. Effect of penicillin and streptomycin on bacterial contamination of chick embryos inoculated with unfiltered sputums. Proc. Soc. Exp. Biol. and Med., 62, 124.
40. Rous, P., and Murphy, J. B. Tumor implantations in the developing embryo: experiments with a transmissable sarcoma of the fowl. J. Amer. Med. Assoc., 56, 741.
41. Murphy, J. B. Transplantability of tissues to the embryo of foreign species. Its bearing on questions of tissue specificity and tumor immunity. J. Exp. Med., 17, 482.
42. Gay, F. P., and Thompson, R. Attempts to cultivate vaccine virus in the growing chick embryo. Proc. Soc. Exp. Biol. and Med., 26, 556.
43. Woodruff, A. M., and Goodpasture, E. W. Susceptibility of chorioallantoic membrane of chick embryos to infection with fowl pox virus. Amer. J. Path., 7, 209.
44. Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J. The cultivation of vaccine and other viruses in the chorioallantoic membrane of chick embryos. Science, 74, 371.
45. Goodpasture, E. W., and Buddingh, G. J. Human immunization with a dermal vaccine cultivated on the membranes of chick embryos. Science, 78, 484.
46. Goodpasture, E. W. Some uses of the chick embryo for the study of infections and immunity. Am. J. Hyg., 28, 111.
47. Burnet, F. M. Influenza virus infection of the chick embryo lung. Brit. J. Exp. Path., 21, 147.
48. Nigg, C., Crowley, J. H., and Wilson, D. E. The use of chick embryo tissues and fluid as antigens in the complement fixation reaction in influenza. J. Immunol., 42, 51.
49. ..... On use of chick embryo cultures of influenza virus in complement fixation tests. Science, 91, 603.
50. Buddingh, G. J., and Polk, A. D. Experimental meningococcus infection of chick embryo. J. Exp. Med., 70, 485.
51. Dawson, J. R. Study of chick embryo-adapted rabies virus. Amer. J. Path., 17, 177.
52. Eichorn, E. A. Technique for the intravenous inoculation of chick embryos. Science, 92, 245.
53. Polk, A., Buddingh, G. J., and Goodpasture, E. W. Experimental study of complement and hemolytic amboceptor introduced into chick embryos. Amer. J. Path., 14, 71.
54. Beveridge, W. I. B., and Burnet, F. M. The cultivation of viruses and rickettsiae in the chick embryo. Spec. Rep. Series Med. Res. Council, London, No. 256.
55. Anonymous. The multiplication of viruses. Vol. III, No. 2, Eli Lilly and Company.
56. Blundell, G. P. A modified technique of cutting the egg shell for virus cultivation. Science, 100, 155.
57. Alexander, R. A. Experimental meningococcus infection of chick embryos. J. Exp. Med., 70, 485.
58. Dunham, W. B. Egg inoculator and shell membrane teaser for virus culture. Science, 95, 609.
59. Himmelweit, F. Observations on living vaccinia and ectromelia viruses by high power microscopy. Brit. J. Exp. Path., 19, 108.
60. Ten Broeck, C., Cultivation of the hog cholera virus. J. Exp. Med., 74, 427.

61. Goodpasture, E. W., and Anderson, K. Virus infection of human fetal membranes grafted on the chorioallantois of chick embryos. Am. J. Path., 18, 563.
62. Hirst, G. K. Direct isolation of human influenza virus in chick embryos. J. Immunol., 45, 293.
63. Francis, T., and Salk, J. E. A simplified procedure for the concentration and purification of influenza virus. Science, 96, 499.
64. Anderson, K. Pathogenesis of *Herpes simplex* virus infection in chick embryos. Amer. J. Path., 16, 137.
65. Bernkopf, H. Cultivation of influenza virus in the chorioallantoic membrane of de-embryonated eggs. Proc. Soc. Exp. Biol. and Med., 72, 680.
66. Rivers, T. M. Viral and Rickettsial Infections of Man, 2nd edition, J. P. Lippincott Company, Philadelphia.

To State Societies of Medical Technologists: If your BULLETIN and Editor are not listed below, please send information to AJMT, 25 Hermann Professional Bldg., Houston 25, Texas.

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Texas, *Newsletter*, John Chubbuck, 206 E. Houston, Tyler.
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Virginia, *Technoscope*, Mrs. Frances Crouch, 3633 Roundhill Avenue, Roanoke
Washington, *Newsletter*, Mrs. Jacqueline Bahrenburg, St. Luke's Hospital, Spokane
West Virginia, *The Microscoop*, Clementia Proudfoot, Myers Clinic, Phillipi
Wisconsin, *The Journal of Wisconsin Association of Medical Technologists*, Mr. Kenneth Smith, St. Luke's Hospital, Milwaukee

EXPERIMENTS WITH COMMERCIAL BEET JUICE AS A COMPONENT OF CULTURE MEDIA*

NANCY LU CONRAD, B.S., M.T. (ASCP)
Bacteriology Laboratory, The Ohio State University Hospital, Columbus, Ohio

The organisms Bacillus subtilis, Escherichia coli, and Staphylococcus aureus were used as a means of studying the properties of various culture medias to which had been added commercial beet juice. The primary purpose of the experiment was to produce a suitable stock culture media by using beet juice as a variant. It can be stated that exclusive of properties of pH there was no great distinction among different varieties of beet juice.

Nutrient agar slants were used as controls in the first phase. Different amounts of beet juice were added to melted nutrient agar slants (tubes) making various percentages of beet juice in the agar slants. All slants were kept for twenty-four hours previous to inoculation to test for sterility. The pH was approximately 6.0-6.2. The slants were then inoculated with the test organisms and checked from six to forty-eight hours (at six-hour intervals) with the result that the organisms on the beet juice slants grew not so rapidly but the growth remained luxuriant longer. By varying the percentage of the beet juice and thereby the pH the media was fairly well adopted to all the test organisms.

By adding sterile human plasma to some of the beet juice agar tubes it was found that the organisms did not grow as quickly or as prolifically as when using the beet juice alone with agar. Of the three test organisms used the gram negative organism responded most satisfactorily to the beet juice enriched media.

The second phase of the experiment dealt with varying the amount of beet juice added to slants composed of beef heart, tryptose, sodium chloride Na_2HPO_4, and agar. If luxuriant growth is desired this is recommended, as growth occurs quickly and fairly heavily. Within 24 hours all organisms had grown out sufficiently to enable an examination by smear to be made. In this case as in the previous one the varying of the pH by increasing or decreasing the beet juice content aided in adopting the media to the various organisms. It was noted that growth lasted longer and remained moister on the slants with the beet juice rather than on the control slants. Close observation showed that growth was excellent for at least three weeks.

* Read before annual Convention A.S.M.T., Louisville, Ky., June, 1953.

The third part of the experiment involves media composed of the following ingredients per liter of solution (distilled water).

Beet juice	12.5 cc.
Peptone	.2 gms.
Dextrose	.5 gms.
Na Cl	1.09 gms.
Na_2CO_3	.5 gms.
Na_2HPO_4	.5 gms.
Agar	16 gms.

Various amounts of beet juice were added to different samples and slants were made.

After testing for sterility the organisms were introduced to the slants. The pH was also varied in order to adapt the media more readily to the organisms.

By close and periodic checking the following observations were made.

1. It was noted that approximately 24 hours elapsed before any apparent growth was noted.
2. In 36 hours reasonable growth for study purposes had taken place.
3. The organisms remained in good condition for about one month before transfers had to be made.
4. The color of the media gradually faded until in about four weeks it had changed from dark rust to medium tan.
5. The media did not begin to dry out or split until about the fourth or fifth week.
6. The organisms grew moderately longer than on the stock culture medias used as controls.

Summary

It was found that commercial beet juice can be used to good advantage in stock culture media in the following ways: By using it to vary the pH of the media without detracting from the nutritive value, by using it to cut down the enrichment value and thus prolong the growing times of various bacteria and by preventing the drying out of the media for a longer period of time.

A SMALL PLASTIC MEASURING DIPPER

BERNADINE HAGAN, M.Sc., M.T. (A.S.C.P.)
South Bend Medical Foundation, Inc., 531 N. Main St., South Bend 1, Ind.

It is occasionally desirable to have a small measuring dipper which will deliver an approximate amount of a powdered material so that one can avoid weighing each portion. Substances which can be measured by this means include barium sulfate for preparing prothrombin free plasma and sodium metabisulfite or sodium hydrosulfite in the preparation of reducing solutions for demonstrating sickling phenomenon in red blood cells.

We have found that such a dipper can be constructed easily from plastic drinking straws. A ½ inch length of straw for the cup and a 4 to 7 inch length for the handle are cut with scissors. One end of the handle is immersed in about ¼ inch of chloroform in a small tube (9x75 mm.) for 50 to 60 seconds. This softens the plastic without completely dissolving it. As soon as the handle piece is removed from the chloroform, the softened end is pinched flat with the fingers and held for ½ minute to form a flat base to which the cup can be fastened. Any plastic adhering to the fingers can be cleaned off later with chloroform. The compressed end of the handle is reimmersed in the chloroform for a few seconds. Then it is removed and one end of the ½ inch piece is quickly pressed into the softened base. It is held firmly between the thumb and finger for ½ minute until it hardens in place.

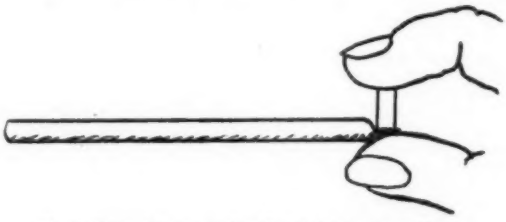

Fig. 1. Fastening cup to handle of plastic straw dipper.

The excess plastic around the base is trimmed off with the scissors so that it will not interfere with accurate measurements.

The dipper is filled with the powder for which it is to be used and then the contents are dumped out and weighed. The top of the cup is trimmed down in proportion to the excess weight. It is rechecked and trimmed until it will deliver the amount desired.

The use of plastic straws for making small measuring dippers has proved a rapid simple procedure. It is easy to adjust the volume to deliver the desired weight of the material for which it is made.

ANNOUNCEMENTS

FACTS AND FIGURES ABOUT THE 1953 Annual Convention of the AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

989 or "11 less than 1000" people attended the annual convention in Louisville, Kentucky. There were 300 from Kentucky (209 from Louisville). 86 from Ohio made the largest "out-of-state" attendance (there were 60 each from Illinois and Indiana). Hawaii had one representative, Canada had 3. 582 members registered, with 78 MT (ASCP)s NOT ASMT members. 48 students and 320 visitors attended. 190 men and 799 women were present. There were 108 Sisters at the meeting.

What will be the increase at Miami Beach in June 1954?

REPORT OF THE PRESIDENT OF THE AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS*

When I accepted the highest office that this Society can offer, I accepted it in good Faith—Faith in you, in the entire membership of ASMT, and in the Board of Directors which had been elected to work with me. I fully realized that the duties and responsibilities of this office would be many, but I knew that with the help of this Advisory Council that the determination to fulfill the obligation of this office in the best interest of the Society could be realized. As a result of our combined efforts, the following report is made possible:

All appointments to Standing committees were made and a complete listing appeared in the July-August issue of the AJMT. Instructions for committee members were mimeographed and distributed to those present at the Portland meeting; those not present in Portland received their instructions by mail. A letter of THANKS for having served on a committee was sent to those retiring from the Chairmanship and to those retiring from the completion of their appointments.

The Oregon Resolution letter brought many inquiries, comments, and votes of confidence in the President of this Society and in the Board of Directors. These were acknowledged whenever possible.

The Whitney questionnaire brought forth another deluge of response, and the wishes of the Council were carefully and seriously noted.

A copy of the Crane proposal on RECLASSIFICATION AND MODIFICATION OF ADMISSION REQUIREMENTS OF APPROVED SCHOOLS was received from an alert ASMT member. Immediately the chairman of the Standards & Studies committee was informed about this proposal. Your President asked that the Council be polled as soon as possible so that we might have available the recommendation of this body concerning the proposal. An invitation was issued by Osborne Brines, M.D., Chairman Executive Committee of the American Society of Clinical Pathologists, to the President of the ASMT to appear and present this Society's views on the Crane Proposal at a hearing on Sunday afternoon, October 12, 1952. Upon the request that one other member be appointed to represent ASMT, exclusive of those on the Board of Registry, your President appointed Miss Ruth Hovde, University of Minnesota School of Medical Technology, and a member of the Public Relations committee of ASMT, as the other ASMT representative. A report of this hearing has been made in the Newsletter. The October meeting of the Board of Registry was attended. The Registry generously set aside the following amounts in the 1952-53 budget for use by the ASMT: Educational Program: $1,000.00; Vocational Guidance and Recruitment: $2,000.00.

Two items for the Agenda of the Board of Registry were presented: 1. that "The Board of Directors of ASMT recommends at the request of

* Read before the Advisory Council, Louisville, Ky., June 14, 1953.

the Advisory Council that the House of Delegates request positive action through the Board of Registry and the Committee of Approved Schools, on the matter of training and position of doctors' aides, and that laboratory aides be defined." It was the opinion of the Board of Registry that no one had been able to state adequately what the duty and training of the aide should be. This will possibly be clarified at the October 1953 meeting. 2. that "One who is facing military duty and is finished with his or her training as a medical technologist be permitted to take his examination before the regular time." The opinion of the Board of Registry indicates that an examinee could take the regular exam no matter where he or she might be at the time the exam was scheduled whether October or May.

In accordance with your wishes concerning the hiring of a Public Relations Counselor, and in complying with the directive of the Board of Directors, Misses Nix, White, Eichman, and I met with Mr. Lewis Glassner of Glassner & Associates on Monday, October 13, 1952, to discuss the possibility of obtaining the services of Glassner & Associates. As a result of this meeting, and after Mr. Glassner's proposal to the Board on what he could and would do for ASMT, the Board of Directors voted to retain Glassner & Associates as the Public Relations counsel for ASMT.

The Will Corporation and the Denver Chemical Corporation were contacted concerning their wish to continue their donation of $100.00 each to the Hillkowitz Memorial Award. Miss Ellen Skirmont, Public Relations Committee chairman, was designated to represent ASMT at the meeting in which the Board of Regents of the American College of Surgeons and the Board of Commissioners of the Joint Commission on Accreditation of Hospitals conveyed the Hospital Standardization Program to the Joint Commission on Accreditation of Hospitals.

A request was received from Henry Hunt, M. D., President of the ASCP for one ASMT member to be appointed to the Film Committee. Miss Ruth Hovde, Public Relations committee, ASMT, was appointed and for two ASMT members to be appointed to the ASCP Committee on Reclassification and Training of Medical Technologists. Mrs. Elsa Kumke, Legislation committee, ASMT, and Miss Anna Bell Ham, Standards & Studies committee, ASMT were appointed.

The Sept.-Oct. and the Nov.-Dec. issues of the American Journal of Medical Technology were guest editored by Colorado and Oklahoma, respectively. The diligence which these two affiliates exercised in editing their respective issues is commendable. The President of this Society was asked to OK the material that went into each issue. This was a responsiblity for which neither the affiliates nor your President were prepared; consequently, upon the recommendation of the Council and the vote of the Board of Directors, the AJMT was put back into the hands of Miss Rose Matthaei who consented to continue as Editor. An Editorial Board of Specialists was set up, and approved, by the Board of Directors.

In order to work more effectively with our public relations counselor in carrying out the internal public relations program he advocated, this officer went to Chicago in November for a conference. The following suggestions were made by Mr. Glassner:

1. To appoint a committee with recognized ability to evaluate the the efficiency of the Executive Office—this in addition to the committee from the Board of Directors appointed to investigate sites for the Executive Office. The following were appointed: Heloise Brown, C.P.A., Houston, Texas—our auditor; Ralph Pelton, Gulf Publishing Company, Houston, Texas—representing our publishers; Mr. Lewis Glassner, Chicago, Ill.—our Public Relations Counselor. Mr. Glassner's report has been sent to the members of the Council. The report of Heloise Brown and Ralph Peltor have been sent to Mr. Glassner.

2. To inform the Council of the contents of these reports before the June meeting IF POSSIBLE.

Mr. C. Patton Steele's report was in the Jan.-Feb. AJMT.

Miss Lucile Harris' report was in the March-April AJMT. Miss Harris was on the original Board Committee to investigate the Office, and was therefore placed on the extra-Board committee to evaluate the office.

3. To direct a letter to the Council requesting qualities, duties, and training expected in an Executive Secretary.

Glassner & Associates agreed to carry out the directive of the Board of Directors by drawing up the written contract for the Executive Secretary. A new ASMT exhibit, designed to educate the physician and the hospital administrator on the qualifications and training of the MT (ASCP) was approved by Mr. Glassner. This has been exhibited at the following meetings: Midwest Hospital Association meeting, Kansas City, Mo., Kansas Society of Medical Technologists—state meeting, Topeka, Kansas; Minnesota Society of Medical Technologists—state meeting in conjunction with the Upper Midwest Hospital Association meeting, Minneapolis, Minn.; the Catholic Hospital Association meeting, Kansas City, Mo.; and the annual meeting of the ASMT, Louisville, Ky.

The American Society of Medical Technologists has been officially represented at the following meetings by the President (in addition to those already mentioned):

Georgia Society of Medical Technologists—April 10, 11, 1953

Midwest Hospital Association meeting—April 17, 1953

Kansas Society of Medical Technologists—April 18, 1953

Met Mary Eichman in Washington, D. C., for meetings with key members of the CS-AS—April 24

D. C. Society of Medical Technologists—April 25, 1953

Catholic Hospital Association meeting—May 23-28, 1953

Kansas City Tech Club dinner meeting—May 27, 1953

Your President-Elect represented the ASMT at the meeting of the Montana Society of Medical Technologists—April, 1953

In addition to the usual communications which go out from this office, the following special ones have been taken care of: letter to the new registrants, letter to go out with the renewals (these are sent to the Registry in Muncie for mailing), letter to the Pathologists to accompany the pre-convention flyers.

A letter soliciting the help of the ASCP Councilors and the President of State Pathologists Societies in the recruitment of more students, and in the opening of more approved schools was signed by Henry F. Hunt, M.D., President, ASCP; David A. Wood, M.D., President, American College of Pathologists, and the President of ASMT.

It is the duty of the President to work closely with the General Chairman of the ASMT convention and with the Program chairman of the ASMT convention. I do not need to remind you that planning and executing a convention of this magnitude is a tremendous job. Everything planned is subject to the approval of the General Chairman and the Chairman of the Board of Directors. This planning covers a period of two years.

Your President has no aims, thought, and ambition other than the aims, thought, and ambition that any Executive Officer has for the progress of the Society he represents. He is obligated to serve to the best of his ability, to serve in accordance with the wishes of the membership as expressed by their representatives in the House of Delegates, and to serve as the Chairman of the Board of Directors. This I have tried to do.

Your attention is called to the following recommendations which are being offered with the thought of knitting this Society closely together in such a strong working unit that mutual interests and a common understanding of our needs can supersede cliques and personalities:

1. for a strong internal public relations program.
2. to invite foreign students to State and ASMT meetings.
3. to set aside in the budget an amount to finance trips of the President, Pres-Elect, and Executive Secretary to cement relationship between the ASMT and its affiliates.
   a. Suggest that the affiliates arrange their schedule of meetings so that the President and the Pres-Elect can take time off in the fall and in the Spring to make visits to as many affiliates as possible in a given period of time (viz., two weeks). Since both officers presumably would be in different geographical areas, this would mean that at least 2/3 of the affiliates could be visited. From experience gained this year in visits to the states, this would greatly stimulate interest in the ASMT and would pay big dividends in affiliate participation in the ASMT in addition to stimulating interest in ASMT membership.
   b. The Executive Secretary is extended many invitations to participate in various functions and to represent the Society at meetings to which Executive Secretaries of a Society are normally invited—this our budget has never provided for.
4. That the Council clear its AGENDA before the annual meeting in the following manner:
   a. Committees appointed from the Council to investigate each item, clear the manner of handling same, and submit in writing the proper manner in which the Council may act upon same. This will facilitate the work of the Council because all avenues of approach to the problem will have been investigated previous to the meeting of the Council.
5. It would follow then that an AGENDA be prepared for the following and sent out at least two weeks ahead of the anticipated meeting of
   a. the Board of Directors, and
   b. the House of Delegates.
6. The States be given a deadline for sending in items for the Agenda for the Board, Advisory Council, or House of Delegates, and that this deadline be rigidly adhered to. The reason for this is that an Agenda can not be prepared well in advance when no items have been submitted, and if not submitted well in advance, there is no time for proper study, investigation, and suggested manner of handling.
7. That the Chairman of all ASMT committees send in progress reports at the time stipulated in their instruction sheet. This information is needed for the proper functioning of this Society if we are to serve the membership in the proper manner, and if we are to function in a businesslike manner.
8. The Council to have a meeting with the newly installed President of this Society so that an understanding of the wishes of each pertaining to the work of that particular year may be accomplished. The responsibilities of Office can be minimized if the Council understands the plans of the President concerning the progress of the Society.
9. The Board of Directors to meet on Friday—post-convention—in order to plan how matters are to be handled that have been relegated to the Board by the House of Delegates, unless definitely stipulated by the House.
10. That the Nomination & Elections committee be shown proper respect. Each year it is increasingly difficult to prepare a good slate for your consideration. The candidates for office are carefully screened—not only for eligibility but for loyalty to ASMT. In any democratic society nominations from the floor are in order unless a mail ballot is used, but it stands to reason that something is wrong when the membership has been given every opportunity to submit candidates for office to the

committee, and for the committee to do all the work necessary in order to submit a good slate, and then to have a complete new proposal submitted from the floor. Such a practice if allowed to continue will mean that the membership will not submit names for the slate and those who are contacted by the committee—though excellent potential—will refuse. The attitude of the membership will be "what's the use? We might as well have nominations from the floor in the first place." We might just as well not have a deadline for submitting the names of candidates for office, we might as well not have a Nominating Committee if we continue something which is considered by many both disrespectful and dangerous.

11. That the services of our public relations firm be utilized to the fullest extent, and that the suggested outline of procedure, recommendations, and advice of our public relations counsel be followed conscientiously.

Respectfully submitted,
/s/ Sadie Cartwright, MT (ASCP) President
1952-53

IN MEMORIAM

As the last issue of the JOURNAL went to press, on July 22, 1953, our Advisory Editor, Dr. A. H. Braden, a pioneer in the field of laboratory medicine, died in St. Joseph's Infirmary, Houston, Texas. Dr. Braden had been pathologist there for nearly twenty-five years and had been an allergist and pathologist in Houston some ten years longer. Those of us who have worked with him closely through the years feel a keen personal loss—his kindly advice, his sincere interest and insight into the problems of medical technologists and medical technology will be missed. A fellow physician said of him some years ago, "Dr. Braden is the living example of one who truly practices according to the oath of Hippocrates." His ability to express in the simplest language that which means most to the laboratory worker is well exemplified in his last message to us, the editorial he wrote for the March-April, 1953, JOURNAL. Let us take his message and "emphasize in our teaching curricula for internes, residents, and medical technologists that the laboratory cannot be used as a short-cut in establishing a diagnosis, but only in its rightful place as a valuable adjunct."

ANNOUNCEMENTS

A.S.M.T. ANNUAL MEETING
The place—Miami Beach, Florida
The time—June 13-17, inclusive, 1954
Headquarters Hotel—The Delano
PLAN NOW TO ATTEND

PROGRAM:

All technologists planning to submit papers to be read before the 1954 convention, with subsequent publication in THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY, should notify the Program Chairman

Miss Anna Bell Ham, MT (ASCP)
1190 South Alhambra Circle
Coral Gables, Flordia

before December 15, 1953.

The awards for which you can compete, and the rules governing them are as follows:

REGISTRY AWARD: Open to any REGISTERED medical technologist (ASCP). The work reported does not have to be original.

ASMT AWARDS: These three awards are open to ASMT members only. The work reported does not have to be original.

PARASITOLOGY AWARD: This $25.00 award is for the best paper on parasitology and is open to ASMT members only.

Contact Miss Ham NOW. Watch subsequent issues of this journal for further rules on how papers will be submitted. Start writing that paper NOW.

Scientific Exhibits

Scientific Exhibits presented by ASMT members and by state societies will be eligible for convention awards. Start to plan yours NOW.

MEDICAL RESERVISTS TO EARN CREDIT POINTS FOR ATTENDING MILITARY SURGEONS MEETING NOV. 9-11, 1953

Reserve credit points may be earned by Medical Service reserve officers on inactive duty for attendance at the daily sessions of the forthcoming 60th annual meeting of the Association of Military Surgeons, the Department of Defense announced today.

This authorization covers eligible physicians, dentists, veterinarians, nurses, women's medical specialists, and Medical Service Corps Officers of the U. S. Army, Navy, and Air Force Reserves.

Rear Admiral Winchell McK. Craig (MC) USNR, President of the Association, will open the meeting at 9 a.m., Monday, November 9, 1953, in the Presidential Ballroom of the Statler Hotel, Washington, D. C., by introducing the senior officers of the various Federal medical and health agencies. The scientific portion of the program will follow and continue through Wednesday, November 11.

Point credits will be awarded to eligible reserve officers on the basis of one point for each day of attendance, provided sessions attended total more than two hours. Each day of the meeting will be considered a session.

Reserve officers will be required to register for each day's session by presenting themselves to representatives of their respective services at the registration desk. Properly authenticated reports of attendance will be rendered to all Army Headquarters, Naval Districts and the Naval Reserve Officers Performance Recording Unit, and numbered Air Force Headquarters to assure creditation.

The meeting will be devoted to subjects having direct military application and will provide an excellent opportunity for Reserve officers to earn Reserve credit points while being brought up-to-date on the latest developments of military medicine.

UROLOGY AWARD

The American Urological Association offers an annual award of $1000.00 (first prize of $500.00, second prize $300.00 and third prize $200.00) for essays on the result of some clinical or laboratory research in Urology. Competition shall be limited to urologists who have been in such specific practice for not more than ten years, and to men in training to become urologists.

The first prize essay will appear on the program of the forthcoming meeting of the American Urological Association, to be held at the Waldorf-Astoria, New York City, May 31-June 3, 1954.

For full particulars write the Executive Secretary, William P. Didusch, 1120 North Charles Street, Baltimore, Maryland. Essays must be in his hands before February 1, 1954.

POSTGRADUATE SHORT COURSES FOR 1953-54 ANNOUNCED

A series of ten short postgraduate courses (in-service training program carried on by the Army Medical Services for Medical Corps officers) designed to help medical officers in outlying installations keep abreast of recent medical advances, aid them in developing and improving techniques and skills, and serve as a means of developing qualified teachers and instructors for all phases of professional education and training within the Army Medical Service, will be conducted during the fiscal year 1953-54, the Office of the Army Surgeon General has announced.

Hospitals, medical centers and other installations participating, will be as follows: Brooke Army Medical Center, Clinical Laboratory Course (March 15-19, 1954); Fitzsimons Army Hospital, Symposium on Pulmonary Diseases (September 21-25, 1953), Clinical Electrocardiography and Recent Advances in Cardiovascular Diseases (November 9-14, 1953); Letterman Army Hospital, Rehabilitation Techniques in Neuromuscular Diseases and Disabilities (April 5-10, 1954); Armed Forces Institute of Pathology, Forensic Pathology (November 2-6, 1953); Pathology of the Oral Regions (March 8-12, 1954); and Walter Reed Army Medical Center, Seminar in Urology (September 7-9, 1953), Symposium on Circulation and Homeostasis (October 5-9, 1953), Symposium on Physical and Mental Health Standards (April 16-17, 1954) and Recent Advances in Medicine and Surgery, including Psychosomatic Aspects of Medicine and Surgery (April 19-30, 1954).

The courses are open to personnel on the basis of their professional training and experience. Chiefs or assistant chiefs of service at hospitals and officers well advanced in their specialty are designated as having level I qualification and are eligible to attend any of the courses.

Officers partially qualified in the specialty, officers with a special interest in the specialty, ward officers and dispensary surgeons responsible for medical treatment in this specialty—Level II qualification—are eligible to attend the courses at Brooke Army Medical Center, Fitzsimons Army Hospital, the Armed Forces Institute of Pathology and the Symposium on Circulation and Homeostasis at Walter Reed Army Medical Center.

Applications for admission to any course must be submitted at least six weeks prior to the opening date. Army medical officers on active duty and full-time Civil Service physicians at Army installations should forward their applications through channels to the Office of the Surgeon General, Department of the Army, Washington 25, D. C., Attention: Personnel Division.

Officers having second priority—not on active duty—should forward their applications through channels to the Army Area Commander to whose jurisdiction they are assigned. National Guard medical officers should apply under current National Guard regulations, forwarding their

applications to the National Guard Bureau, Washington 25, D. C. Other armed forces medical officers should apply through channels.

In submitting requests for admission to the courses, applicants should indicate: (1) Their name, rank, service number and primary and secondary M.O.S., (2) organization and station, (3) title of course desired, (4) list of all other courses they have attended during the past two years with dates of each, and (5) category and expiration date.

FROM THE NATIONAL SOCIETY FOR MEDICAL RESEARCH

Research scientists, in order to assure reliable scientific results, maintain rigid standards for the care of the animals they use, according to a comprehensive report on this subject appearing in the current issue of the BULLETIN of the National Society for Medical Research. There are standards for housing, feeding, disease control and equipment.

The article is based on a survey of animal care practices, regulations and proposals. No similar compilation has been made before.

Viewed from the standpoint of minimum standards, quarters for laboratory animals are considered adequate if they meet four simple basic requirements—cleanliness, proper light, adequate ventilation and sufficient and proper heating. However, most animal quarters tend toward elaborateness rather than this basic simplicity, the survey revealed. Pictures which illustrate the article indicate that few structures are more elaborate than some of the newer buildings which house laboratory animals. In one of the newest medical schools animal quarters cost five times more per cubic foot to construct and equip than any other part of the structure. In many medical centers, the only air conditioned rooms are those in which the animals are housed. Glazed tile walls and quarry tile floors are commonplace in medical animal quarters.

Of the four minimal standards, cleanliness is the most important, and must be achieved regardless of the plant itself. Cleanliness demands daily hosing of quarters, proper drainage of floors, and walls constructed of vermin excluding material.

Strict dietary standards are maintained, especially for puppies and animals that are used in long term research projects. At one typical institution mentioned, dogs are fed fresh bone, coarse ground lean horse meat, 100 per cent whole wheat bread, supplemented with vitamins, minerals and 3 per cent steamed bone meal.

Since animals, like human beings, are subject to a wide variety of infectious diseases, great precautions must be observed in laboratories to control or eliminate any possibility of infection. Obviously, an extraneous infection could invalidate a whole research project. It is routine to innoculate against distemper, infectious hepatitis, and like animal diseases.

While more and more animal quarters are being placed under the direct supervision of a veterinarian, the animal attendant under him is of great importance. This attendant, who is closely concerned with the welfare of the animals, can make or break an important research project. He must be more than a mere caretaker, and must have rudimentary scientific knowledge in addition to a liking for animals.

In all animal laboratories throughout the country, codes governing the use and treatment of animals are prominently posted. The code, adopted by the University of California is reproduced in the article in its entirety. In addition to the self-imposed codes of the profession, there are various state laws regarding the care of laboratory animals. Many of the laws providing that unclaimed, otherwise doomed animals, in public pounds be saved for research use contain provisions for the care of laboratory animals. Other states have written similar provisions into their state public health codes or cruelty to animals statutes.

HEMATOLOGY SHORT COURSE

The University of Minnesota will present a course in Hematology for Medical Technologists at the Center for Continuation Study on October 6-7, 1953. This year's course is designed to emphasize technical aspects of hematology of use to the medical technologist. The program will be presented under the direction of Dr. Gerald T. Evans, Director of Hospital Laboratories, and the faculty will include members of the staff of the University of Minnesota Medical School. For further information write: The University of Minnesota, The Medical School, Department of Continuation, Minneapolis 14, Minnesota.

OLD JOURNALS NEEDED

The AMERICAN JOURNAL OF MEDICAL TECHNOLOGY needs copies of the first issues of the BULLETIN of the American Society of Clinical Laboratory Technicians:

Volume 1, Nos. 1 (November 1934) and
1a (January 1935)

as well as copies of THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY:

Volume 2, No. 6 (November 1936)

and a limited number of

Volume 11, No. 3 (May 1945)

Volume 12, No. 1 (January 1946)

Volume 16, Nos. 5 and 6 (September and November 1950)

Volume 18, Nos. 4 and 6 (July and November 1952)

Volume 19, No. 1 (January 1953).

Please write to AJMT, 25 Hermann Professional Bldg., Houston 25, Texas, if you would like to sell your copies at $1.00 each. Do not ship copies until notified by this office.

UNESCO GIFTS

Public libraries throughout the country are providing information on the Unesco Gift Coupon Plan which supplies educational, scientific, and cultural supplies and equipment where they are needed abroad. This informational program is sponsored by the International Relations Board of the American Library Association because many organizations look to public libraries for program materials.

Local libraries are displaying exhibits of photographs and informative materials which explain to voluntary groups how the Unesco Gift Coupon Plan establishes direct relationships between donors and recipient groups in the free countries. Equipment furnished through the plan ranges from laboratory equipment and supplies to vocational and handicraft tools, and audio-visual materials. The library is not selling the coupons. These may be obtained direct from the **Unesco Gift Coupon Office, United Nations, New York.**

The Unesco Gift Coupons in effect are a form of international currency and their use eliminates the purchasing, packing and shipping of materials which often encumber voluntary international assistance. Unesco field science officers in seven parts of the world, together with other Unesco experts and educational leaders in each country, locate the projects to receive assistance. Organizations should specify the type of assistance they wish to give through their Gift Coupons.

COMMITTEE ON CONSTITUTION AND BY-LAWS

The following Article in regard to Amendments to the By-Laws of the American Society of Medical Technologists was adopted at the convention in Louisville, Kentucky, June 17, 1953. "Section 1. The By-Laws of this Society may be amended as follows: (a) any proposed amendment to the By-Laws recommended by either a constituent society or by an active or life member of this Society shall be submitted in writing to the Chairman of the Constitution & By-Laws Committee *not later than December 1. Six copies of the proposed amendment shall be submitted.* The Constitution and By-Laws Committee shall make such changes as may be necessary to provide proper form without altering the meaning of the amendment." Preliminary copies of the By-Laws as revised have been sent to the Presidents of constituent societies and as soon as the entire revision has been printed, a copy will be sent to each member of the society. Send proposed amendments to Rose Hackman, MT (ASCP), Chairman, 4200 E. 9th St., Denver, Colorado.

COMMITTEE ON NOMINATIONS AND ELECTIONS

The Nominations and Elections Committee is looking for your suggestions for the offices to be filled at the next annual meeting of the House of Delegates of the American Society of Medical Technologists. The offices to be filled are: President-elect, Recording Secretary, Treasurer, two members of the Board of Directors, and one member of the Board of Approved Schools.

This is your Society and your chance to pass along the suggestions for candidates for these offices. Don't forget to give the qualifications, previous offices held in state and national societies, and any other pertinent information about the proposed candidates. They must be members in good standing for at least three years prior to election, or active or life members of this Society, and be willing to serve if elected.

Please send seven copies of the qualifications of your proposed candidates to the chairman not later than November 15, 1953.

Ruth Feucht, MT (ASCP), Chairman
1142 East 55th St.
Chicago 15, Illinois

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